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Lipase Immobilization on Ionic Liquid-Modified Magnetic Nanoparticles: Ionic liquids Controlled Esters Hydrolysis at Oil–Water Interface

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Ester hydrolysis at oil–water interface by lipase covalently immobilized on ionic liquid-modified magnetic nanoparticles was investigated. Magnetic supports with a diameter of 10–15 nm were synthesized by covalent binding of ionic liquids (chain length C_4 and C_8 and anions Cl^-, BF_4^-, and PF_6^-) on the surface of Fe_3O_4 nanoparticles. Lipase was covalently immobilized on Fe_3O_4 nanoparticles using ionic liquids as the coupling reagent. Ionic liquid-modified magnetic nanoparticle-grafted lipase preferentially located at the oil–water interface. It has higher catalytic activity than its native counterpart. A modified Michaelis–Menten model was used to elucidate the effect of stirring rate, aqueous–organic phase ratio, total amount of enzyme, and ester chain length. The influences of these conditions on esters hydrolysis at oil–water interface were consistent with the introduction of the ionic liquids interlayer. Ionic liquids could be used to control the oil–water interfacial characteristics during lipase catalyzed hydrolysis, and thus control the behavior of immobilized lipase. *AIChE J,* 58: 1203–1211, 2012
Keywords: magnetic nanoparticles, ionic liquids, lipase, oil–water two-phase, ester hydrolysis

Introduction

Lipase (glycerol ester hydrolase, EC 3.1.1.3) has been used as the "workhorse" in different organic reactions for the synthesis of fine chemicals, pharmaceuticals, etc.[1,2] The biological function of lipase is to catalyze the hydrolysis of ester because the glyceride backbones of lipase are naturally designed to cleave fatty acids for lipolysis in the organism. Hydrolysis is the principal first reaction for production of free fatty acids, which can be transesterified or converted into high-value fatty alcohols.[3] Enzymatic hydrolysis can be performed under mild conditions when comparing with the conventional methods for oil hydrolysis. So, it is more advantageous for producing high value and heat sensitive fatty acids.[4]

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Industrial application of lipase for the splitting of fats and oils is promising as an energy-saving process, especially for producing high value products or thermosensitive fatty acids.[5,6]

Lipase-catalyzed hydrolysis takes place at the oil–water interface between the aqueous phase containing the enzyme and the oil phase. Enzymatic reactions are greatly influenced by the availability of the interface. One reason is that enzyme has to penetrate the interface as a first step in the reaction.[7,8] The other reason is that the intrinsic specific activity of enzyme is significantly higher at the interface than in the bulk solution, which is "interfacial activation."[9] Thus oil–water interface becomes a key point of lipase-catalyzed hydrolysis. Surfactant was used in some researches to form emulsion for increasing interfacial area, but surfactant contamination and product purification remain to be serious problems.[7] Many other attempts such as lipid coated lipase,[10] lipase filled reversed micelles,[11] and lipase entrapped membrane[12] have also been proposed to increase the catalytic efficiency, but the deactivation of enzyme and the difficulty to separate products and catalyst preclude the wide application in industry.[2]

Ionic liquids are nonvolatile liquid salts and stable at room-temperature. They are widely used in extraction, catalysis, and other scientific applications.[13,14] Recent researches have confirmed that ionic liquids are suitable media for enzyme catalysis.[15,16] It is also reported that ionic liquids consist of a charged hydrophilic head group and a hydrophobic "tail" domain, so ionic liquids have surface active properties similar to amphiphiles or surfactants[17] and the properties can be tuned by changing the structure of cations and anions. All these advantages make ionic liquids potential materials for enzyme immobilization to control the catalysis at the oil–water interface.

In this study, functionalized ionic liquids [C_n(A)C_4(-D)Im]X (1-butyraldehyde-3-(carbonic acid)-imidazolium salt) were used as the coupling reagent for immobilizing biocatalyst on magnetic particles (Scheme 1). Magnetic particles allow the convenient recovery of catalysts from products solution after reaction, so high concentration of enzyme can be used without consideration of the solvent pollution and high cost. The ionic liquids have one arm of carbonic acid group, which is chemically grafted on the surface of magnetic particles, and the other arm is linked with lipase through butyraldehyde group. Ionic liquids controlled oil–water interfacial hydrolysis was investigated. To our knowledge, this is the first time that ionic liquids have been used as the linker between enzyme and supports and applied to control enzyme reaction at oil–water interface.

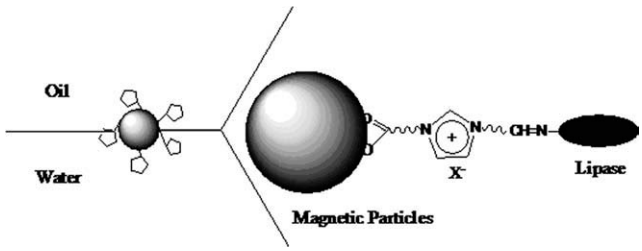
Methods

Materials

Candida rugosa lipase was purchased from Sigma-Aldrich Company. The catalyst was used without further purification. All ester substrates used were prepared by condensations from the corresponding acid chlorides and alcohols as conventional methods. All other chemicals were purchased from commercial suppliers and were of the highest purity available.

Synthesis of magnetite nanoparticles

The magnetite nanoparticles were prepared by the conventional coprecipitation method.[18] $FeCl_3.6H_2O$ (2.33 g) and $FeCl_2.4H_2O$ (0.86 g) were dissolved in 100 mL deionized water under nitrogen gas with vigorous stirring at 90°C. Ten milliliter of 25% $NH_3.H_2O$ was added to the solution. The magnetite precipitates were washed several times with deionized water and once with 0.02 mol L^{-1} sodium chloride by magnetic decantation.



Scheme 1. Lipase loaded on the magnetic particles using ionic liquids interlayer (X=Cl^-, BF_4^-, PF_6^-).

Synthesis of butyraldehyde-substituted imidazole

Butyraldehyde-substituted imidazole was synthesized according to the procedure described.[19] Sodium hydride (4.00 g, 100 mmol, 60% suspension in mineral oil) was washed with dry pentane under nitrogen. The reaction flask was placed in an ice bath and THF (30 mL) was added. Imidazole (6.23 g, 91 mmol) was dissolved in 30 mL of freshly distilled THF, and the solution was slowly added into the reaction flask under nitrogen atmosphere. The reaction mixture was allowed to stir for 2–4 h before being removed from the ice both. Then 4-chlorobutanal diethyl acetal (91 mmol) was added dropwise. The reaction mixture was refluxed for 4–6 h, filtered, concentrated under vacuum, washed with fresh THF, and filtered again. After THF was removed under vacuum, the liquid residue was dissolved in 100 mL ether and washed with water. The resultant liquid was distilled under reduced pressure through a 15-cm Vigreux column to give the butanal diethyl acetal-substituted imidazole as a light yellow liquid. The butanal diethyl acetal substituted imidazole was hydrolyzed using 3 mol L^{-1} HCl water solution as the catalyzer under nitrogen at 50°C for 6 h. The product was extracted with ether for five times, and ether was removed in vacuo to get butyraldehyde-substituted imidazole with a yield of 40%.

Preparation of ionic liquid-modified magnetic nanoparticles

Fe_3O_4 (100 mg) was dispersed in 100 mL ethanol and sonicated for 30 min. One gram 7-chlorounoctanoic acid or 3-chlorobutyric acid was added to the solution.[20] The system was treated with microwave irradiation under nitrogen for 15 min. After reaction, the solid was isolated by a magnet and washed with acetonitrile (100 mL) twice and methanol (100 mL) twice. Nucleophilic substitution using butyraldehyde-substituted imidazole was achieved by heating the reaction materials to 60°C under nitrogen for 48 h. The particles were washed with ethanol for several times to get the ionic liquid-modified magnetite nanoparticles. The desired anion

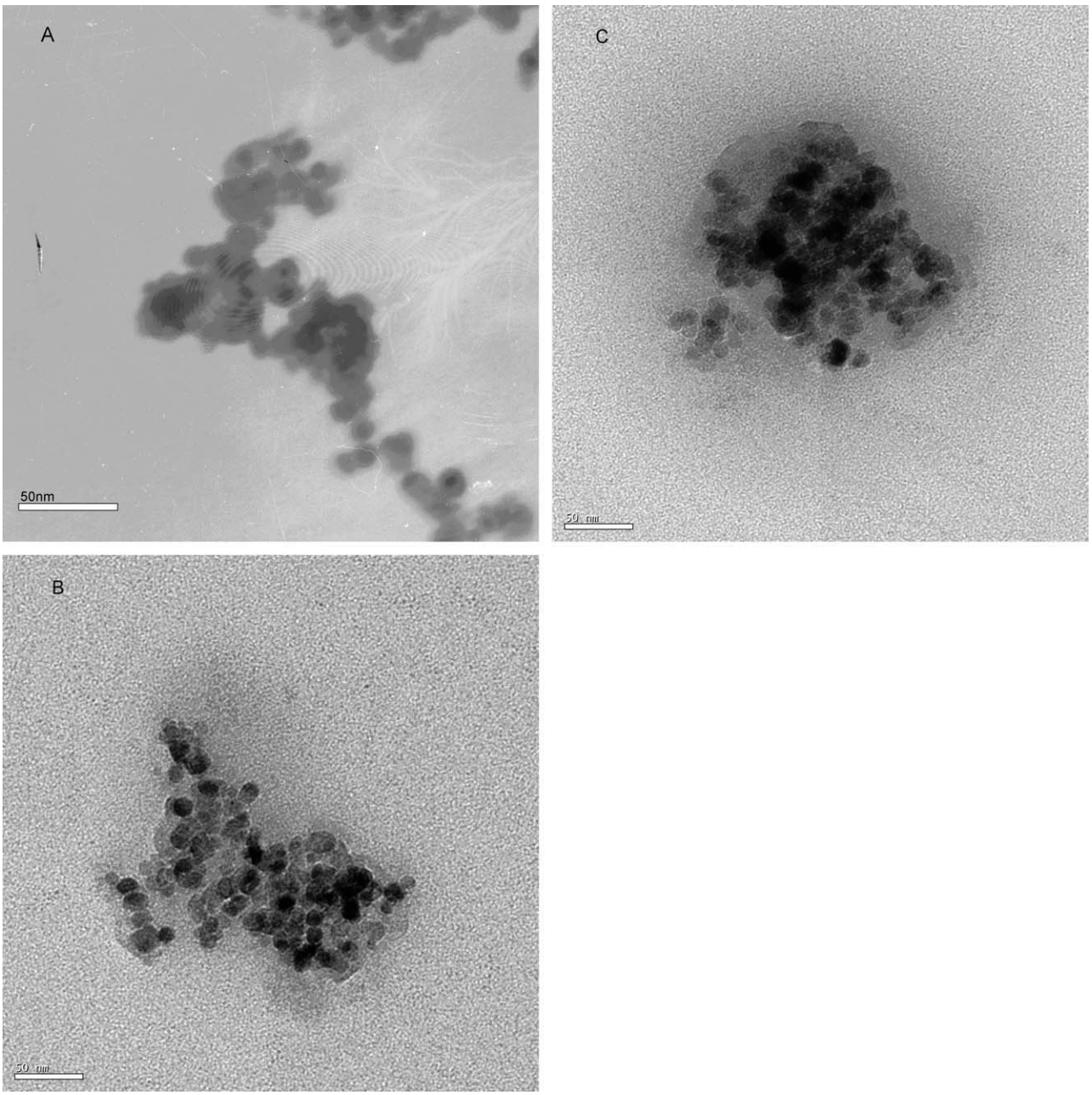


Figure 1. The TEM images of (A) naked Fe_3O_4 particles; (B) Fe_3O_4 particles grafted with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ interlayer; (C) Fe_3O_4 particles modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ interlayer and lipase (the bars are 50 nm in all pictures).

of ionic liquids (BF_4^- and PF_6^-) was obtained by ion exchange with anion salt according to Mehnert et al.[21] The particles were washed with water for several times.

Covalent immobilization of lipase on ionic liquid-modified magnetic nanoparticles

Twenty milligrams lipase was dissolved in 15 mL phosphate buffer (0.1 mol L^{-1}, pH 7.0). Fifty milligrams support was added followed by gently shaking the system at 25°C for 6 h.[20,22] The unbounded enzyme was removed using a magnet and the precipitates were washed carefully with buffer and water for several times. The protein concentration of supernatant before and after the immobilization steps was measured by Bradford's dye binding assay.[23]

Ester hydrolysis in oil–water two-phase system

$$C_{n-1}H_{2n-1}COOCH_2CH_3 + H_2O \xrightarrow{\text{Lipase}} C_{n-1}H_{2n-1}COOH + CH_3 + CH_2OH \quad (1)$$

Ester hydrolysis (Eq. 1) in two-phase system was performed according to Wu et al.[24] Ionic liquid-grafted lipase (containing

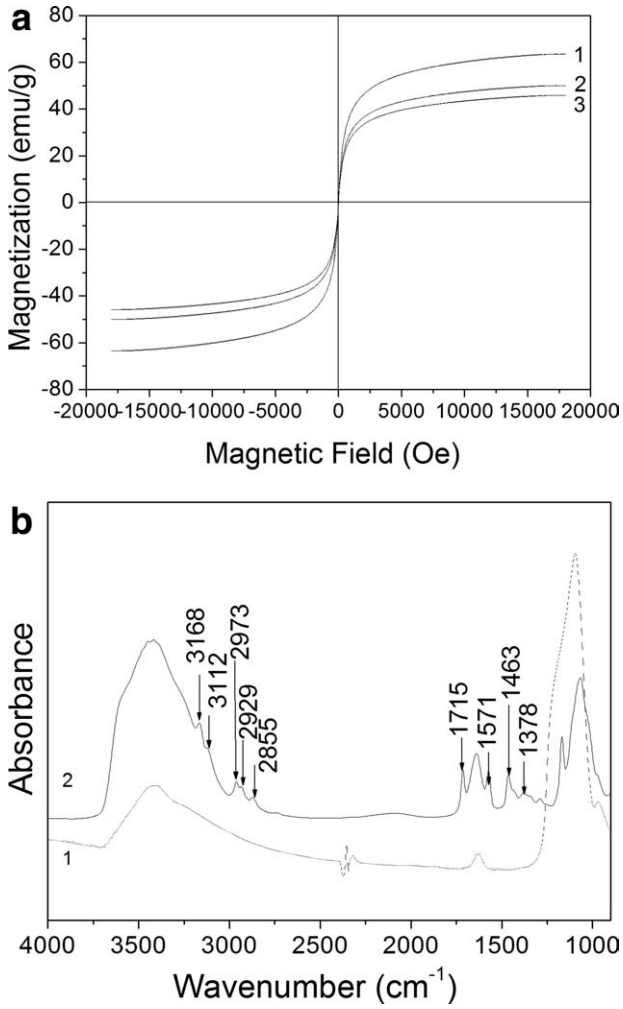


Figure 2. a: The AGM of Magnetic particles (1-Fe_3O_4; 2-Fe_3O_4 modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ interlayer; 3-Fe_3O_4 modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ and lipase). b: FTIR spectrum of 1-Fe_3O_4; 2-Fe_3O_4 modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$.

1 mg of protein) and ethyl dodecanoate (2 mmol) were added into 20 mL isooctane and 10 mL 0.05 mol L^{-1} phosphate buffer (pH 7.8) mixture. The reaction was kept at 40°C under stirring for 30 min, and the immobilized enzyme was magnetically concentrated. The amount of produced acid was measured by titration using 0.01 mol L^{-1} KOH ethanol solution. Each reaction was repeated for three times. The kinetic constants of free and immobilized lipase were determined based on Michaelis–Menten equation.[7,25]

Characterization of ionic liquid-modified magnetic nanoparticle-grafted enzyme

FTIR spectra were recorded on Bruker Vecter 22 FTIR spectrometer with a deuteriotriglycine sulfate detector and a resolution of 2 cm^{-1} using KBr pellets. The size and morphology of the particles were observed by transmission electron microscopy (TEM; PHILIPS, FEI TECNAI20). A drop of sample was placed on a carbon-coated 200 mesh copper grid, followed by drying the sample at ambient conditions before it was attached to the sample holder on the microscope. Magnetic characterization was performed on an alternating gradient magnetometer (MicroMag^{TM} 2900) at room temperature with applied field of −18,000 to 18,000 Oe.

Results and Discussion

Characterization of Fe_3O_4 particles modified with ionic liquids and lipase

Figure 1 shows the TEM images of magnetic particles without modifier, with ionic liquids interlayer, and with ionic liquids and lipase, respectively. It is found that the diameter of naked Fe_3O_4 particles is about 12 nm, which is smaller than the critical size (25 nm) of superparamagnetism. When magnetic nanoparticles are grafted with ionic liquids, the diameter is about 15 nm. The diameter of nanoparticles modified with ionic liquids and lipase is about 15–20 nm. Aggregation is observed. This may be due to the small size and huge surface energy of the magnetic nanoparticles.

Figure 2A shows the magnetic response of Fe_3O_4 particles, Fe_3O_4 particles modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ interlayer, and Fe_3O_4 particles modified with ionic liquids $[C_4(A)C_4(D)Im]BF_4$ and lipase. It is found that all systems are superparamagnetic, which implies that the magnetite-loaded lipase could be recovered and reused with a magnet. The saturation magnetization of naked magnetite is 63 emu g^{-1}, whereas the saturation magnetization of the ionic liquid-grafted particles reduces to 49 emu g^{-1}. The Fe_3O_4 particles modified with ionic liquids and lipase have a saturation magnetization of 47 emu g^{-1}. This result suggests that about 4% weight fraction of enzyme is modified on the nanoparticles, which is consistent with the result of Bradford's dye binding assay. The magnetic catalyst responded very rapidly to a magnetic field, and left a clear supernatant in less than 10 s.

The IR spectrum (Figure 2B) of ionic liquid-modified Fe_3O_4 shows the characteristic bonds of ionic liquids. The peaks at 3168 cm^{-1} and 3112 cm^{-1} correspond to C—H stretching vibrations of the imidazolium ring. The peaks at 2973 cm^{-1}, 2929 cm^{-1}, and 2855 cm^{-1} represent the stretching vibrations of —CH_3 and —CH_2, while 1463 cm^{-1} and 1378 cm^{-1} correspond to their bending vibrations. The peaks at 1715 cm^{-1} and 1571 cm^{-1} refer to the stretching and bending vibrations of C=O in ionic liquids, respectively. FTIR spectra prove that magnetic nanoparticles are bonded with ionic liquids.

Enzyme enrichment at the oil–water interface

The enzyme enrichment at the oil–water interface with ionic liquids layer are validated in Figure 3. It is found that ionic liquid-modified magnetic nanoparticle enzyme distributes in aqueous bulk phase at initial 10 s (Figure 3A), but they gradually concentrate at the oil–water interface (Figure 3B). The residual concentration of enzymes in continuous aqueous phase is negligible at full equilibrium. The phenomenon can be explained as follows. On one hand, ionic liquids have surface active properties. They can adsorb at a surface of gas, liquid, or solid with the hydrophilic ends of the

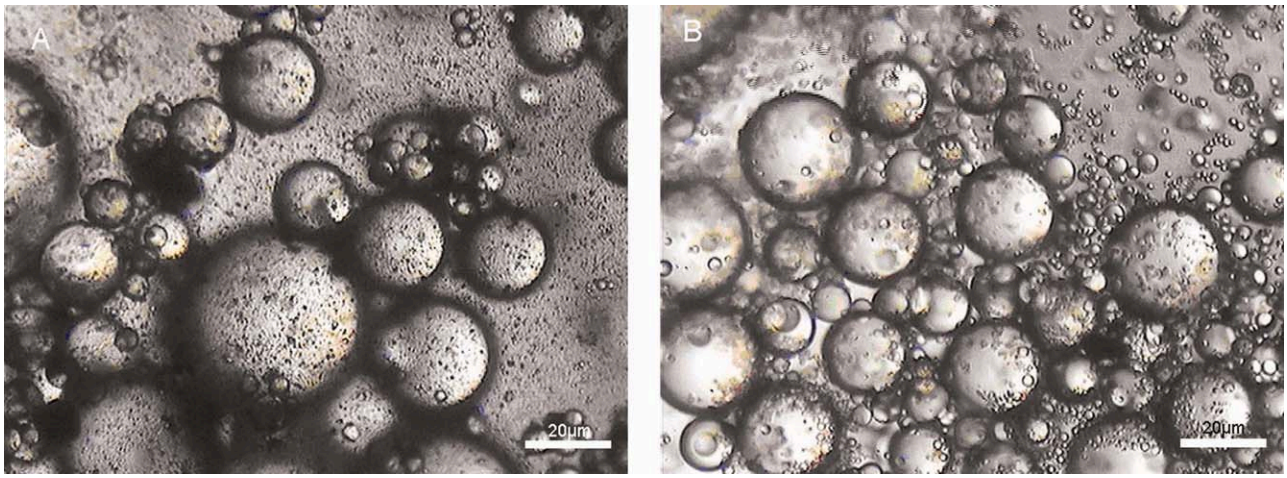
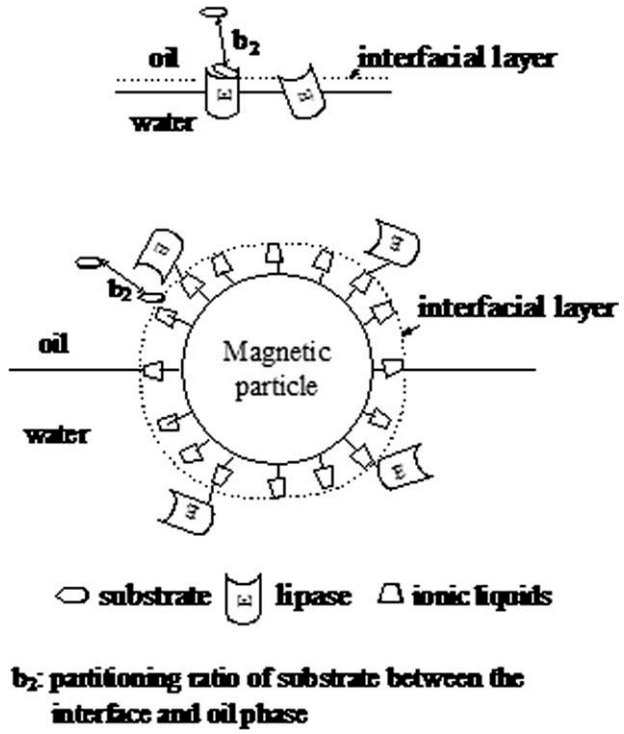


Figure 3. Optical micrographs of oil/water emulsion in the presence of ionic liquids modified nanoparticles-lipase system (A: 10 s; B: 30 s) [[C_4(A)C_4(D)Im]BF_4, 20 mL isooctane, 10 mL 50 mmol/L phosphate buffer, 1 mg lipase].

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

molecules oriented toward the aqueous phase.[17] On the other hand, nanoparticles preferentially locate at ionic liquids/water interface, which stabilizes the ionic liquids/water emulsion in similar ways as that of surfactants.[26,27]

As a result, in the ionic liquid-modified magnetic nanoparticles–lipase system, the oil–water two-phase system transfers into oil–ionic liquids–water pseudo-three-phase system. Scheme 2 illustrates the mass equilibrium of the substrate between the interface and organic phase in native and ionic liquid-modified nanoparticles immobilized lipase system. In native enzyme system, the parameter b_1 represents the partitioning ratio of enzyme between the interface and water phase, whereas b_2 represents the partitioning ratio of substrate between the interface and oil phase. In the ionic liquid-modified nanoparticle-grafted lipase system, b_1 represents the distribution ratio of enzyme between the ionic liquids interlayer and water phase, whereas b_2 refers to the mass ratio of substrate between the ionic liquids interlayer and organic phase in pseudo-three-phase system.



Scheme 2. Mass equilibrium of substrate between the interface and organic phase in native and ionic liquids grafted lipase systems.

Effect of ionic liquids hydrophobicity on enzymatic activity at interface

Figure 4 correlates the slope of the initial catalytic rate v_0 with log P of ionic liquids. Log P is the logarithm of the partition coefficient measuring partitions of ionic liquids between aqueous and organic phase. It serves as an indicator

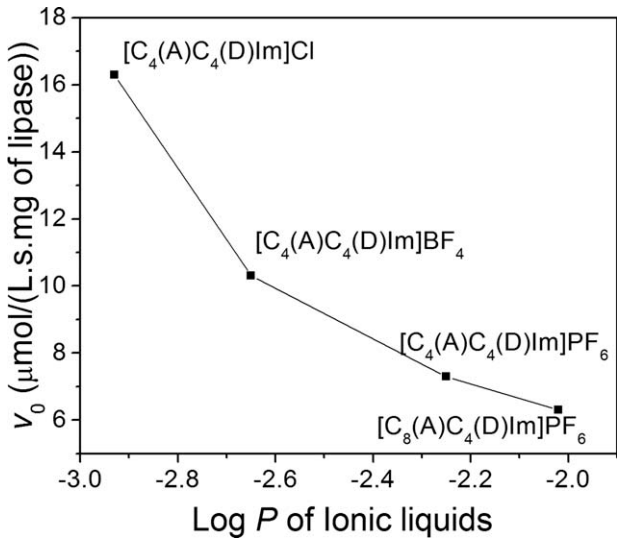
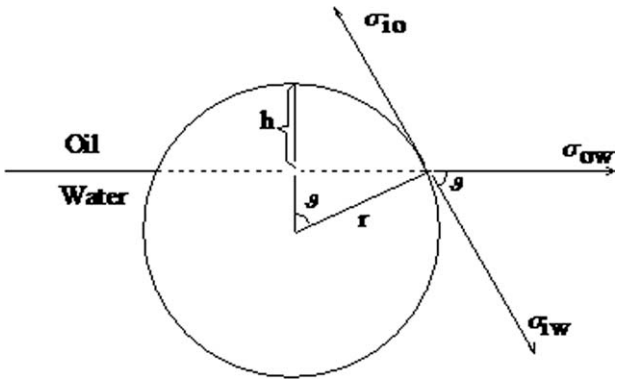


Figure 4. Dependence of v_0 on log P of ionic liquids.



Scheme 3. Contact angle θ in the magnetic particles/water/oil system.

of hydrophobicity of ionic liquid.[15] It is found that the initial catalytic rate v_0 decreases inversely with log P of ionic liquids. Increasing the hydrophobicity of ionic liquids from $[C_4(A)C_4(D)Im]Cl$ to $[C_4(A)C_4(D)Im]BF_4$ (the hydrophobicity of ionic liquids immobilized on the magnetic nanoparticles is slightly higher than its counterpart of the bulk solvent), v_0 decreases from 16.3 to 10.4 μmol $L^{-1}s^{-1}mg^{-1}$ (of lipase). It decreases a litter further when increasing the hydrophobicity of ionic liquids from $[C_4(A)C_4(D)Im]PF_6$ to $[C_8(A)C_4(D)Im]PF_6$. Lipase grafted with hydrophilic ionic liquids, such as $[C_4(A)C_4(D)Im]Cl$ and $[C_4(A)C_4(D)Im]BF_4$, could get better performance than that grafted with hydrophobic ionic liquids.

When ionic liquids are introduced as the bridge between lipase and magnetic nanoparticles, the oil–water two-phase system has been transferred into oil–ionic liquids–water pseudo-three-phase system. According to Young's equation,[28] the contact angle θ of magnetic particles at the liquid/liquid interface is determined by:

$$\cos\theta = \frac{\sigma_{io} - \sigma_{iw}}{\sigma_{ow}} \tag{2}$$

where σ_{io}, σ_{iw}, and σ_{ow} represent the interfacial tension between ionic liquids–oil, ionic liquids–water, and oil–water, respectively (where the σ_{io} and σ_{iw} refer to the interfacial tension between ionic liquids bounded on the magnetic particles and the aqueous or organic bulk phase. The tension is slightly different from the interfacial tension between the bulk phases; Scheme 3). By increasing the hydrophobicity of ionic liquids, the interfacial tension between ionic liquids and water σ_{iw} increases (while σ_{io} keeps nearly constant), which results in the increase of contact angle θ and more volume of the magnetite-particle immersing into the organic phase. It is well-known that the enzyme in organic solvent has much lower activity than that in water.[29] Therefore, lower catalytic velocity is obtained in more hydrophobic ionic liquids.

Effect of stirring rate, phase ratio, and total amount of enzyme on enzymatic activity at interface

Figure 5A shows the effect of stirring rate on enzymatic hydrolysis activity in native and ionic liquid-modified magnetic nanoparticle-lipase systems. It is found from Figure 5A that v_0 in ionic liquid-grafted lipase system is generally larger than that of native enzyme. v_0 increases proportionally with stirring rate in native lipase system, while in ionic liquid-grafted lipase system, it maintains relatively stable in a wide range of stirring rate.

Figure 5B presents the relationship between v_0 and the aqueous–organic phase ratio in native and ionic liquid-modified magnetic nanoparticle–lipase systems. In native lipase system, v_0 increases rapidly at small phase ratio and drops sharply when the phase ratio exceeds 0.5. In hydrophobic ionic liquid-modified magnetic nanoparticle–lipase system, v_0 increases with phase ratio initially. It remains constant above the phase ratio of 0.5. In hydrophilic ionic liquid-modified system, v_0 increases with phase ratio initially, then it slightly decreases when the phase ratio is larger than 0.5. The maximum value of v_0 is hydrophilic ionic liquids system > hydrophobic ionic liquids system > native lipase system.

Figure 5C illustrates the influence of the total enzyme amount E_t on the enzymatic hydrolysis activity in native and ionic liquids–lipase systems. It is found that the catalytic rate of ionic liquid-grafted lipase system increases proportionally with E_t in isooctane/water system. While in native lipase system, it reaches the saturation value at sufficiently large E_t.

The different behavior in native and ionic liquid-grafted lipase systems can be interpreted by the distribution behavior of enzyme in the two-phase system. It was found that K_m was not influenced by the experimental conditions such as stirring rate, phase ratio, and total amount of enzyme, so according to the derivation of Refs. [7] and [25] and:

$$V_m/E_t = k_2/(1 + V_w/b_1A) = k_2/(1 + R/(3b_1)) \tag{3}$$

where k_2 is the rate constant from ES complex to product. b_1 represents the mole ratio of enzyme at the interface to that in bulk aqueous phase. A, E_t, V_w and R refer to the total interfacial area, total amount of enzyme, volume of aqueous phase, and drop volume-surface mean radius, respectively.

Native lipase distributes evenly between the interface and bulk water phase, which leads to a small b_1.[7] Therein, the drop volume-surface mean radius R in Eq. 3 plays an essential role in determining the enzymatic velocity. According to Calderbank's observation, R in oil–water two-phase system is dependent on the stirring rate and aqueous–organic phase ratio:[30]

$$R = 4.08D[1 + 3.75\alpha/(\alpha + 1)]/(D^3N^2\rho/\sigma)^{0.6} \tag{4}$$

where D, N, ρ, and σ are the impeller diameter, the rotation speed of the impeller, the density of the continuous phase, and the surface tension of the dispersed phase, respectively. α is the phase ratio between water and organic solvent. Equation 4 suggests that rapid stirring rate N or small phase ratio α often leads to small R value, which facilitates the improvement of catalytic velocity in native lipase system (Figures 5A, B). On the other hand, large phase ratio α facilitates the accumulation of native lipase in the aqueous phase, which results in low effective concentration of native lipase at the interface,[7] leading to low catalytic velocity in two-phase system.

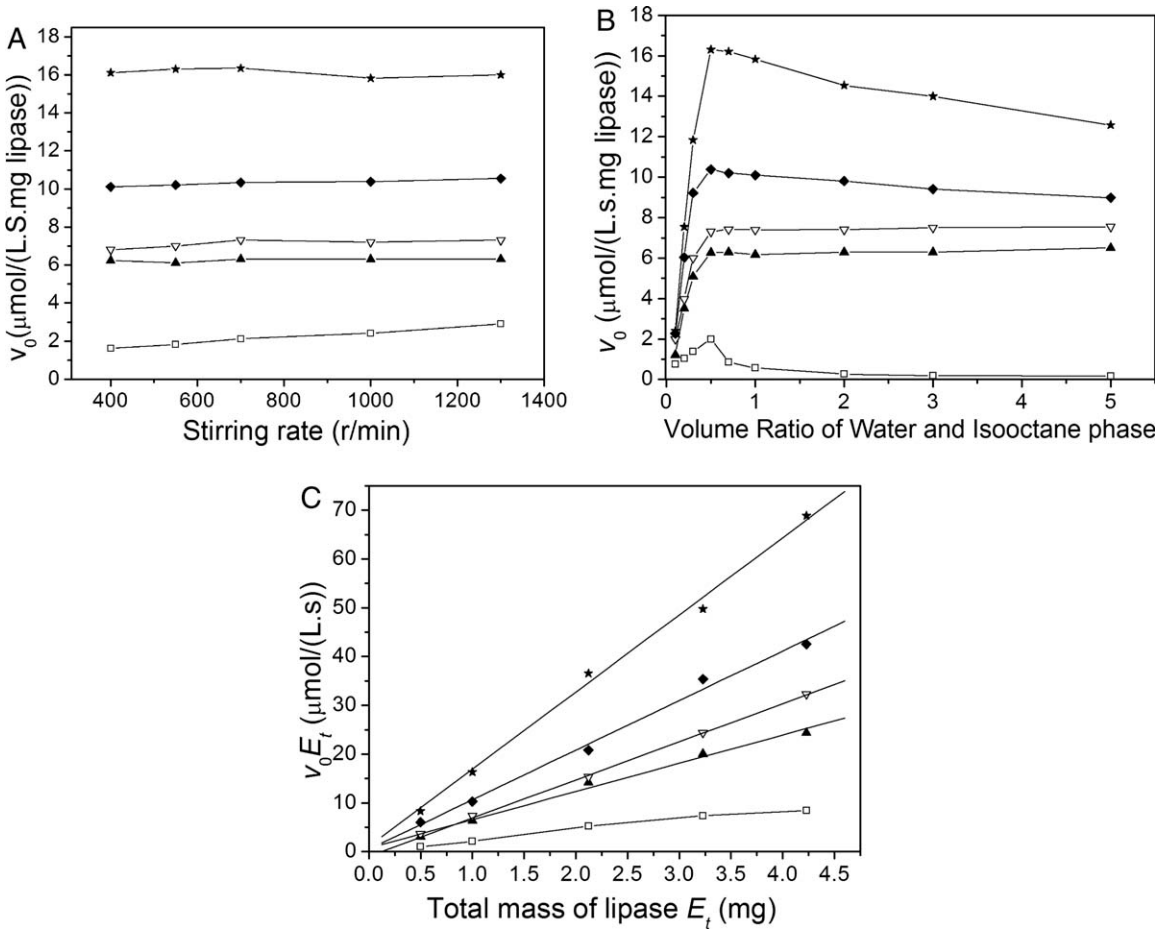


Figure 5. A: Effect of stirring rate on v_0 in native and ionic liquids modified nanoparticle-lipase systems [20 mL isooctane, 10 mL 50 mmol/L phosphate buffer at pH 7.8, 1 mg lipase] (□: Native lipase; ▲: $[C_8(A)C_4(D)Im]PF_6$; ▽: $[C_4(A)C_4(D)Im]PF_6$; ◆: $[C_4(A)C_4(D)Im]BF_4$; ★: $[C_4(A)C_4(D)Im]Cl$). B: Effect of aqueous-organic phase ratio on v_0 in native and ionic liquids modified nanoparticle-lipase systems [20 mL isooctane, 1-100 mL 50 mmol/L phosphate buffer at pH 7.8, 1 mg lipase, 700 r/min] (□: Native lipase; ▲: $[C_8(A)C_4(D)Im]PF_6$; ▽: $[C_4(A)C_4(D)Im]PF_6$; ◆: $[C_4(A)C_4(D)Im]BF_4$; ★: $[C_4(A)C_4(D)Im]Cl$). C: Dependence of initial activity on the mass of lipase E_t in native or ionic liquid grafted lipase system [20 mL isooctane,10 mL 50 mmol/L phosphate buffer, pH 7.8, 700 r/min] (□: Native lipase; ▲: $[C_8(A)C_4(D)Im]PF_6$; ▽: $[C_4(A)C_4(D)Im]PF_6$; ◆: $[C_4(A)C_4(D)Im]BF_4$; ★: $[C_4(A)C_4(D)Im]Cl$).

In the ionic liquid-grafted lipase system, the situation is quite different. Ionic liquid-grafted lipase locates at the oil–water interface, which results in large parameter b_1 in Eq. 3. As a result, Eq. 3 can be further translated into:

$$V_m/E_t = k_2 \tag{5}$$

The two parameters E_t and k_2 in Eq. 5 are independent of the stirring rate and phase ratio, so the catalytic velocity in ionic liquid-grafted lipase system keeps constant irrespective of the two parameters, which is in line with the experimental results in Figures 5A, B. The rapid increase of catalytic velocity at phase ratio smaller than 0.5 can be attributed to the improved substrate diffusion at the oil–water interface.[25] The decrease of catalytic velocity in hydrophilic ionic liquid-grafted lipase system at large phase ratio may be due to the leaching of hydrophilic anion from the ionic liquids interlayer, which destroys the enzymatic conformation and leads to low enzymatic activity.

In ionic liquid-grafted lipase system, the relationship between V_m and E_t can be got from Eq. 5,

$$V_m = E_t k_2 \tag{6}$$

According to Eq. 6, V_m is proportional to E_t in ionic liquid-grafted lipase system, while in native enzyme distributes evenly between the oil–water interface and aqueous bulk phase. As a result, the adsorbed amount Γ of enzyme at the interface would reach a saturation value at sufficiently large E_t according to Langmuir isotherm, which leads to a constant catalytic velocity in oil–water two-phase system.

Effect of substrate specificity

Figure 6A presents the effect of acyl chain length on the apparent Michaelis constant K_m^1. It shows that K_m^1 decreases with the acyl length of ester before the critical value of the acyl length. After that, K_m^1 increases again. For native lipase

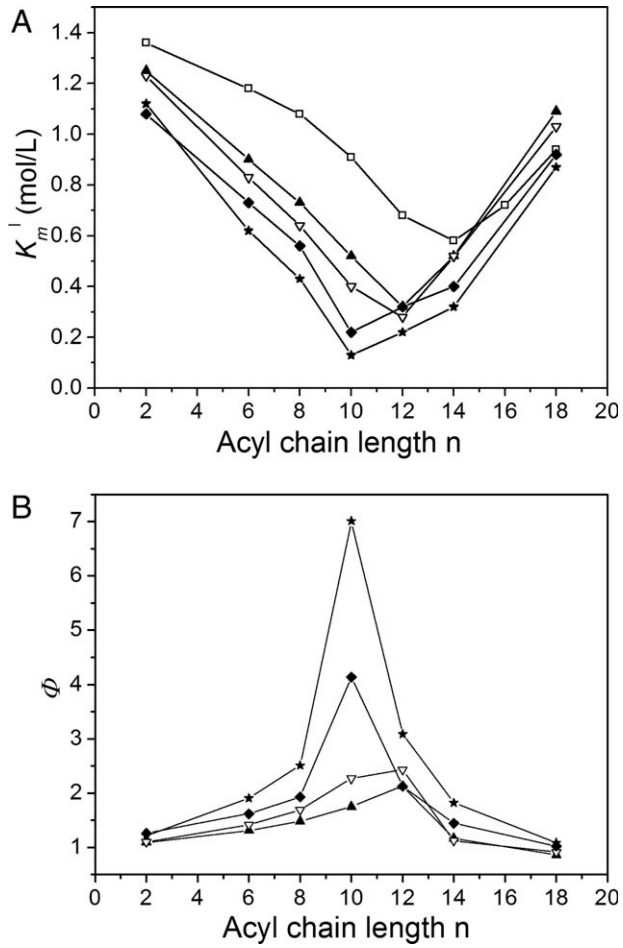


Figure 6. A: Effect of acyl chain length n of ethyl esters on the K_m^1 in native and ionic liquids-lipase systems [20 mL isooctane, 10 mL 50 mmol/L phosphate buffer at pH 7.8, 1-200 mmol/L easter, 1 mg lipase, 700 r/min] (□: Native lipase; ▲: [C_8(A)C_4(D)Im]PF_6; ▽: [C_4(A)C_4(D)Im]PF_6; ◆: [C_4(A)C_4(D)Im]BF_4; ★: [C_4(A)C_4(D)Im]Cl). B: Effect of acyl chain length n of ethyl esters on magnified ratio Φ in native and ionic liquids-lipase systems (▲: [C_8(A)C_4(D)Im]PF_6; ▽: [C_4(A)C_4(D)Im]PF_6; ◆: [C_4(A)C_4(D)Im]BF_4; ★: [C_4(A)C_4(D)Im]Cl).

system, this critical value is 14 carbon atoms. For ionic liquids [C_8(A)C_4(D)Im]PF_6 or [C_4(A)C_4(D)Im]PF_6 system, it is 12 carbon atoms, whereas for ionic liquids [C_4(A)C_4(D)Im]BF_4 or [C_4(A)C_4(D)Im]Cl system, it is 10 atoms. The binding ability ($1/K_m^1$) at the optimal length increases of native system < [C_8(A)C_4(D)Im]PF_6 < [C_4(A)C_4(D)Im]PF_6 < [C_4(A)C_4(D)Im]BF_4 < [C_4(A)C_4(D)Im]Cl. The result suggests that the introduction of ionic liquids interlayer facilitates the improvement of the binding ability of substrate ($1/K_m^1$) in oil–water two-phase system.

To compare with native lipase system, magnified ratio Φ for K_m^1 is introduced, which refers to the enrichment of substrate concentration at the oil–water interface with introduction of ionic liquids interlayer. Φ can be calculated from Eq. 7:

$$\phi = K_m^{\mathrm{N}}/K_m^{\mathrm{IL}} = b_2^{\mathrm{IL}}/b_2^{\mathrm{N}} \quad (7)$$

where K_m^{N} and K_m^{IL} represent the apparent binding constants of native and ionic liquid-grafted lipase. The parameters b_2^{IL} and b_2^{N} refer to the mole ratio of substrates between the interface and organic bulk phase, where the interface represents the oil–water interface in native lipase system and the ionic liquids interlayer for magnetite grafted lipase.

Figure 6B gives the magnified ratio Φ. It is found that ionic liquids interlayer favors the accumulation of short-chain substrates at the oil–water interface. About twofold enrichment of ester concentration is observed in hydrophobic ionic liquids grated lipase system than that of native enzyme. In hydrophilic ionic liquids-enzyme system, 4–7 fold enrichment is obtained. It has been reported that the polarity of ionic liquids based on imidazolium cation was shown to be comparable with that of the lower alcohols such as methanol.[31,32] The high polarity of ionic liquids favors the compatibility of polar substrates, leading to a high hydrolysis rate of short-chain esters at the interface.

Conclusion

This study investigated ionic liquids controlled ester hydrolysis at oil–water interface by immobilized lipase. Functionalized ionic liquids [C_n(A)C_4(D)Im]X (1-butyraldehyde-3-(carbonic acid)-imidazolium salt) were synthesized and used as linker for immobilizing lipase on magnetic nanoparticles. Ionic liquid-grafted enzyme exhibited several advantages over the native lipase. Ionic liquids interlayer created a new pseudo-three-phase different from the conventional oil–water biphase system, and ionic liquid-grafted lipase preferentially resided at the interface. Moreover, the hydrolysis behavior of immobilized lipase could be controlled by ionic liquids. The enzymatic activity increased with increasing the hydrophilicity of ionic liquids, it showed little dependence of the stirring rate and aqueous–organic phase ratio when the phase ratio is above 0.5, and it increased proportionally with the total mass of enzyme. The ionic liquids environment favored the accumulation of polar esters at the ionic liquids interlayer. The optimal acyl chain length decreases with increasing the hydrophilicity of ionic liquids (14 carbon atoms for native lipase, 12 carbon atoms for hydrophobic ionic liquids system, and 10 carbon atoms for hydrophilic ionic liquids systems). Two- to sevenfold enrichment of the substrates was obtained in the ionic liquid-grafted lipase system than that of native enzyme.

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