Hydrophobic Surface Induced Activation of *Pseudomonas cepacia* Lipase Immobilized into Mesoporous Silica

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Supporting Information

**ABSTRACT:** Lipase from *Pseudomonas cepacia* (PCL) was successfully immobilized into siliceous mesocellular foams (MCFs) with various hydrophobic/hydrophilic surfaces. The catalytic performances of immobilized PCL were investigated using the transesterification reaction and hydrolytic reaction as model reactions. The specific activity of immobilized PCL greatly increased with enhanced surface hydrophobicity of MCFs, mainly because of lipase activation via hydrophobic interaction between alkyl groups in MCFs and the surface loop (so-called “lid”) of PCL. Conformational changes of immobilized PCL were further investigated using time-resolved fluorescence spectroscopy with Trp as an intrinsic probe. When the immobilized PCL was suspended in phosphate buffer, short-lived $\tau_1$ shortened and the fractional contribution of $\tau_1$ significantly increased with the increasing level of surface hydrophobicity of MCFs. These results revealed that Trp(s) of the immobilized PCL were surrounded by a hydrophilic microenvironment because of the fact that the opened “lid” permitted the diffusion of water to the active site cleft. However, for the immobilized PCL suspended in n-hexane, long-lived $\tau_1$ increased with the increase of surface hydrophobicity of MCFs. The reduced interaction between Trp(s) and the surrounding protein matrix was due to intercalation of n-hexane into the active site cleft when the lipase was in open conformation. The above results demonstrated that PCL immobilized into MCF with hydrophobic surfaces were in an activated open conformation.

1. INTRODUCTION

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are one of the most frequently used biocatalysts in asymmetric organic synthesis because they have broad substrate specificity and high enantioselectivity with good resistance to organic media. A representative example is lipase from *Pseudomonas cepacia* (PCL), which shows high catalytic activity and enantioselectivity for the resolution of chiral primary and secondary alcohols. Insight into the catalytic process, an outstanding feature of PCL, is the phenomenon termed “interfacial activation”. Ser87-His286-Asp264 catalytic triad in PCL is buried under a closed hydrophobic surface loop (so-called “lid”) in PCL. Conformational changes of immobilized PCL were investigated using time-resolved fluorescence spectroscopy with Trp as an intrinsic probe. When the immobilized PCL was suspended in phosphate buffer, short-lived $\tau_1$ shortened and the fractional contribution of $\tau_1$ significantly increased with the increasing level of surface hydrophobicity of MCFs. These results revealed that Trp(s) of the immobilized PCL were surrounded by a hydrophilic microenvironment because of the fact that the opened “lid” permitted the diffusion of water to the active site cleft. However, for the immobilized PCL suspended in n-hexane, long-lived $\tau_1$ increased with the increase of surface hydrophobicity of MCFs. The reduced interaction between Trp(s) and the surrounding protein matrix was due to intercalation of n-hexane into the active site cleft when the lipase was in open conformation. The above results demonstrated that PCL immobilized into MCF with hydrophobic surfaces were in an activated open conformation.

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synthesis) are commonly used to prepare organic functionalized mesoporous siliceous materials for lipase immobilization. The highest catalytic activity of lipase-based hybrid macroporous foams obtained through integrative chemistry has been reported by Bruin et al. In our previous work, PCL was immobilized into magnetic siliceous mesoporous foam (MCF) functionalized with octyl groups and the resultant biocatalyst exhibited excellent performance in the resolution of 1-phenylethanol. Further, we found that the hydrolytic activities of PCL immobilized into siliceous MCFs were increased with an increasing content of residual template in the mesopores. However, the risk of lipase deactivation is higher in an absolutely hydrophobic microenvironment because the necessary water layer of the protein molecule would be destroyed. Moreover, the incompatibility of a rigid microenvironment and flexible protein molecules would lead to structural changes of protein. To date, conformational changes of protein after immobilization have been rarely reported.

Although “interfacial activation” has a positive effect on the catalytic activity of immobilized lipases, it still lacks direct evidence. Fourier transform infrared (FTIR) spectroscopy, circular dichroism (CD), and fluorescence spectroscopy are the most common methods to study protein structure change induced by a surrounding environment. However, few of them are effective for lipase immobilized into mesoporous silicas, especially when the protein loading is very low, because the background signals from the support severely overlap or mix with protein signals. A time-resolved fluorescence measurement is a useful technique to study the global conformational changes of confined protein because the tryptophan fluorescence is sensitive to the location and interactions within the protein. To date, conformational changes of the immobilized lipase that were associated with fluorescence decay of protein have been rarely reported.

In the present work, PCL was immobilized into siliceous MCFs with various hydrophobic/hydrophilic surfaces. Subsequent evaluation of the catalytic activity of immobilized PCL helps to search for the relation between the support surface property and lipase conformation. Moreover, time-resolved fluorescence spectroscopy was further used to characterize the global conformational changes of immobilized PCL. The influence of surface hydrophobicity/hydrophilicity of MCFs on PCL conformation and function was revealed.

2. EXPERIMENTAL SECTION

2.1. Materials. Amano lipase PS from PCL (30 units/mg) was purchased from Sigma-Aldrich. This product was diluted with dextrin and had a protein content of 2 wt% (determined by the Bradford assay using bovine serum albumin as a standard). Lipase B Candida antarctica (recombinant from Aspergillus oryzae, 10.8 units/mg) and triblock copolymer pluronic P123 (EO20PO70EO20, Mw = 8000) were purchased from Sigma-Aldrich. Phenethyltrimethoxysilane (Ph) was purchased from Gelest. 3-n-Octyl triethoxysilane (C8), 3-aminopropyltriethoxysilane (NH2), tetraethoxysilane (TEOS), and triacetin were obtained from Alfa Aesar. Tributyrin and DL-1-phenylethanol were purchased from Acros Organics and Avocado Research Chemicals, Ltd., respectively. N-Tri-methoxysilylpropyl-N,N,N-tri-n-butylammonium chloride (TBNCl) and N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMNCl) were purchased from ABCR GmbH & Co. KG (Germany). Vinyl acetate and n-hexane were obtained from Shanghai Chemical Reagent Company of the Chinese Medicine Group and pre-equilibrated using sodium pyrophosphate before use.

2.2. Characterization. Scanning electron microscopy (SEM) was performed using a FEI Quanta 200F scanning electron microscope at an acceleration voltage of 20 kV. Transmission electron microscopy (TEM) was recorded on a FEI Tecnai G2 Spirit at an acceleration voltage of 120 kV. Nitrogen sorption isotherms were measured at 77 K on an ASAP 2000 system in the static measurement mode. Before measurements, samples were outgassed at 120 °C for 6 h. The adsorption of water and benzene vapors was measured at 273 K on a Hiden Isochema intelligent gravimetric analyzer after the samples were outgassed at 80 °C for 6 h. Infrared (IR) spectra were recorded on a Thermo-Nicolet Nexus 470 FTIR spectrometer. Samples doped with a proper amount of KBr were pressed into self-supporting wafers and mounted inside an IR cell with a BaF2 window for FTIR spectroscopy. Before measurement, MCF samples and immobilized PCL were heated in the cell under vacuum for 1 h at 100 °C and 2 h at 60 °C, respectively. To evaluate the surface hydrophobicity/hydrophilicity, MCF samples were first pressed into flakes under 6 MPa, then the contact angles formed between sessile water droplets, and the flakes were measured using a contact angle measuring system JC 2000A. The immobilized PCL (20 mg) was suspended in phosphate buffer (NaH2PO4/NaHPO4, at pH 8.0, 50 mM) or n-hexane (2 mL) with stirring for time-resolved fluorescence measurement. Time-resolved fluorescence spectra were recorded on a FS920 fluorescence spectrometer (Edinburgh Instruments, U.K.) using the time-correlated single-photon counting (TCSPC) method. The excitation source was a 285-nm picosecond pulsed light-emitting diode with a pulse width of 833 ps. Decays were collected at 327 nm. For data analysis, commercial software by Edinburgh Instruments was used. The data were fitted using a reconvolution method of the instrument response function (IRF).

2.3. Preparation of Functionalized MCFs. Spherical siliceous MCF was synthesized according to the procedure described by Ying et al. Generally, P123 co-polymer (~ 8 g) was completely dissolved in an acidic solution (20 mL HCl and 130 mL H2O), and then 1,3,5-trimethylbenzene (6.8 mL) was added. The resulting solution was stirred vigorously at 40 °C for 4 h. TEOS (18.4 mL) was added, and huge amounts of white solids were generated immediately. After 5 min of stirring, the above milky suspension was aged for 20 h under a static condition. NH4F solution (92 mg in 10 mL of water) was added to the above suspension, which was then transferred to an autoclave for aging at 100 °C for 24 h. The white precipitate obtained was then filtered, washed with water and ethanol, and dried overnight. The template was removed via calcination of samples in air at 550 °C for 6 h.

Before modification, as-synthesized MCF (1.0 g) was dehydrated at 120 °C for 6 h under vacuum. Organosilane (2 mL) in dry toluene (50 mL) were then added to the above MCF. Pyridine (0.5 mL) was also added to accelerate the reaction between organosilane and surface silanol groups. The above mixture was refluxed under Ar for 24 h, then filtered, washed thoroughly with toluene and ethanol, and dried in a vacuum oven at 60 °C overnight. The sample was denoted as MCF—R (R = NH4F, TMNCl, TBNCl, C8, and Ph), where R is the functional group of organosilane.

MCFs modified with different amounts of octyl group were also prepared, which were denoted as MCF—C8 (L), where L is the grafted amount of octyl group (mmol g−1).

2.4. PCL Immobilization. Functionalized MCF (100 mg) was added to 1 mL of enzyme solution containing crude PCL (100 mg) in 50 mM phosphate buffer (pH 8.0). Before shaking, ethanol (200 μL) was added to increase the impregnation of MCF—R in aqueous media. The suspension was shaken mildly at 4 °C for 12 h to achieve the adsorption equilibrium. After protein adsorption, the suspension was centrifuged and 100 μL of the supernatant was removed from the adsorption system to check for residual activity with the tributyrin assay. The residual mixture was directly homogenized and lyophilized at 10 Pa
and \(-50^\circ C\). The resultant biocatalyst was denoted as PCL/MCF–R. The lipase loading was expressed as “milligrams of PCL per gram of support” and calculated by mass balance of the initial activity loaded for immobilization and the residual activity recovered in the supernatant. Unmodified MCF (100 mg) was suspended in enzyme solution (1 mL) containing crude PCL (50 mg) to achieve highly efficient loading, and 200 \(\mu\)L of ethanol was also added to normalize the adsorption condition. The resultant biocatalyst was denoted as “PCL/MCF”.

2.5. General Procedure for the Non-aqueous Transesterification Reaction. In the reaction, racemic 1-phenylethanol (1.0 mmol) and vinyl acetate (2.5 mmol) were dispersed in \(n\)-hexane (4 mL) at 37 \(^\circ\)C. After 5 min of stirring, PCL/MCF–R (9.9 mg) was added sequentially to initiate the reaction. To compare the catalytic performance quantitatively, the amount of PCL/MCF was normalized to 15.2 mg based on the active protein. Homogeneous catalysis was carried out with 5 mg of crude PCL under the same conditions. The reaction was roughly monitored on thin-layer chromatography and stopped until the conversion reached around 15%. Enantiomeric excess (ee %) and conversion were determined on an Agilent 6890 gas chromatograph with a HP-Chiral-20B column (0.25 mm × 30 m × 0.25 \(\mu \)m). The oven temperature was kept at 110 \(^\circ\)C for 40 min. The injector (split 1:50) temperature was 250 \(^\circ\)C, and the flame ionization detector (FID) temperature was 280 \(^\circ\)C. The flow rates of the carried gases (nitrogen, hydrogen, and air) were 10, 40, and 400 \(\text{mL min}^{-1}\), respectively. The specific activity of immobilized PCL was defined as the amount of produced ester (micromoles) per gram of crude PCL per minute.

2.6. General Procedure for Hydrolytic Reaction. The hydrolytic activity of lipase in the tributyrin system was investigated by measuring the liberation amount of butyric acid according to the method described by Cao et al. A mixture of tributyrin (400 \(\mu\)L) and phosphate buffer (25 mL, pH 8.0, 50 mM) were stirred vigorously in a vessel for 5 min at 37 \(^\circ\)C. Then, PCL/MCF–R (5 mg) or PCL/MCF (7.5 mg) was added to initiate the reaction. The pH of the reaction was kept at 8.0 all along according to the consumption of 0.1 M NaOH. In addition, a blank experiment without added enzyme was carried under the same conditions. The specific activity of immobilized PCL was defined as the amount of produced butyric acid (micromoles) per gram of crude PCL per minute.

The injector (split 1:50) temperature was 250 \(^\circ\)C, and the flame ionization detector (FID) temperature was 280 \(^\circ\)C. The flow rates of the carried gases (nitrogen, hydrogen, and air) were 10, 40, and 400 \(\text{mL min}^{-1}\), respectively. The specific activity of immobilized PCL was defined as the amount of produced ester (micromoles) per gram of crude PCL per minute.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Properties of Siliceous MCFs with Surface Modification. Siliceous MCF, which is composed of large spherical cells interconnected with narrow windows, was selected as a parent support to immobilize PCL. SEM micrographs (panels a and b of Figure 1) show that MCF particles consist primarily of about 5 \(\mu\)m spheres. A higher magnification SEM micrograph (Figure 1c) and TEM micrograph (Figure 1d) show that MCF possesses a mesoporous structure with a diameter of 36 nm. Nitrogen sorption isotherms of the MCF series are typical of type IV with obvious large hysteresis, which in conjunction with corresponding pore size distributions can further support the mesoporous structure of MCF (see Figure S1 of the Supporting Information). The changes of textural properties of MCFs with surface modification are summarized in Table 1. Although surface areas, pore volumes, and pore sizes of functionalized MCFs are decreased in comparison to those in the parent MCF, they are still large enough to accommodate lipase and substrates.

Figure 2A shows FTIR spectra of various MCFs with and without surface modification. The spectra between 4000 and 1500 cm\(^{-1}\) were enlarged by a factor of 10 because the signals of grafted alkyl groups were weaker than that of Si–O–Si. The typical peak at 3747 cm\(^{-1}\) in the spectrum of MCF assigned to the O–H stretching vibration confirms the existence of silanol groups on the surface of MCF. After surface modification, that peak decreases or even disappears because of consumption of silanol groups during the silylation process. All of the functionalized MCFs contain typical peaks between 3100 and 2800 cm\(^{-1}\) assigned to the C–H stretching vibration in alkyl groups. Two new peaks around 3302 and 3369 cm\(^{-1}\) assigned to the N–H stretching vibration prove the grafting of amine groups. Peaks between 3100 and 3010 cm\(^{-1}\) assigned to the C–H stretching vibration and the peak at 1605 cm\(^{-1}\) belonged to the C–C stretching vibration are characteristics of the phenyl group of MCF–Ph. FTIR spectra confirm the surface modification of MCFs with various organic groups. Further, the grafted amounts of organic groups were determined by thermogravimetric analysis based on their weight losses between 120 and 600 \(^\circ\)C, which is in the range of 0.25–1.70 mmol g\(^{-1}\) (Table 1). FTIR spectra of...
the immobilized PCL series (Figure 2B) are dominated by strong dextrin peaks at approximately 3300—3500 cm⁻¹ assigned to the O–H stretching vibration and at 2928 cm⁻¹ assigned to the C–H stretching vibration. The signals originated from alkyl groups have overlapped with the strong response of dextrin. In the enlarged FTIR spectra (Figure 2B, right), the peak at 1660 cm⁻¹ assigned to the C=O stretching vibration of amide group roughly indicates the presence of lipase.

To evaluate the surface hydrophobicity/hydrophilicity of functionalized MCFs, contact angles between sessile water droplets and the material flakes were measured and the results are summarized in Figure 3. Without modification, MCF gives a water droplet contact angle of 25.5° because of its hydrophilic surface property. MCF modified with aminopropyl (MCF–NH₂) becomes a little bit more hydrophobic but still exhibits hydrophilic property with a contact angle of 35.5°. MCF–TMNCl and MCF–TBNCl exhibiting amphiphilic property give contact angles of 42.0° and 56.5°, respectively. The grafted octyl groups increase the hydrophobic property of MCF greatly and give a water contact angle of 81°. The largest contact angle of 111.5° among the samples tested is observed for MCF–Ph. Therefore, the surface hydrophobicity decreases in the order of MCF–Ph > MCF–C8 > MCF–TBNCl > MCF–TMNCl > MCF–NH₂ > MCF according to contact angle measurements.

The surface hydrophobicity/hydrophilicity of representative functionalized MCFs were also evaluated by water and benzene vapor adsorption experiments (Table 2). The amount of adsorbed water and benzene increases with the increase of the relative pressure (P/P₀). At P/P₀ of 0.90, the molar ratio of adsorbed water/benzene is 1.28, 0.67, 0.16, and 0.06 for MCF, MCF–TMNCl, MCF–C8, and MCF–Ph, respectively. It can be concluded that MCF and MCF–TMNCl have hydrophilic and amphiphilic surface properties, respectively. MCF–C8 and MCF–Ph have hydrophobic properties, which are in accordance with the results of contact angle measurement.

<table>
<thead>
<tr>
<th>samples</th>
<th>surface area (m² g⁻¹)</th>
<th>pore volume (cm³ g⁻¹)</th>
<th>pore size (nm)</th>
<th>window size (nm)</th>
<th>grafted amount (mmol g⁻¹)</th>
<th>lipase loading (mg/g)</th>
<th>contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF</td>
<td>438</td>
<td>2.28</td>
<td>36.1</td>
<td>17.9</td>
<td>0</td>
<td>9.6</td>
<td>35.5</td>
</tr>
<tr>
<td>MCF–NH₂</td>
<td>328</td>
<td>2.00</td>
<td>34.2</td>
<td>16.9</td>
<td>1.70</td>
<td>19.2</td>
<td></td>
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<tr>
<td>MCF–TMNCl</td>
<td>306</td>
<td>1.77</td>
<td>29.4</td>
<td>15.6</td>
<td>1.13</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>MCF–TBNCl</td>
<td>318</td>
<td>1.74</td>
<td>29.3</td>
<td>15.5</td>
<td>0.71</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>MCF–C8 (0.25)</td>
<td>359</td>
<td>1.99</td>
<td>33.1</td>
<td>16.0</td>
<td>0.25</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MCF–C8 (0.47)</td>
<td>340</td>
<td>1.96</td>
<td>32.9</td>
<td>15.9</td>
<td>0.47</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MCF–C8 (0.69)</td>
<td>325</td>
<td>1.89</td>
<td>32.6</td>
<td>15.7</td>
<td>0.69</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MCF–Ph</td>
<td>317</td>
<td>1.88</td>
<td>31.0</td>
<td>15.8</td>
<td>0.97</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*The Brunauer–Emmett–Teller (BET) surface area. The sizes of the pore and window were calculated from the adsorption and desorption branches of the nitrogen sorption isotherms using the Barrett–Joyner–Halenda (BJH) method. The weight ratio of crude PCL/MCF was 0.5.*

**Table 1. Comparison of the Textural Parameters, Grafted Amount of Organic Groups, and Lipase Loading of MCFs with and without Surface Modification**

**Figure 2.** (A) FTIR spectra of (a) MCF, (b) MCF–NH₂, (c) MCF–TMNCl, (d) MCF–TBNCl, (e) MCF–C8, (f) MCF–Ph. (B) FTIR spectra of (a) crude PCL, (a) PCL/MCF, (b) PCL/MCF–NH₂, (c) PCL/MCF–TMNCl, (d) PCL/MCF–TBNCl, (e) PCL/MCF–C8, (f) PCL/MCF–Ph.

**Figure 3.** Comparison of specific activities of PCL immobilized into MCFs with various hydrophobic/hydrophilic surfaces for the non-aqueous transesterification reaction of racemic 1-phenylethanol. (Square dot: contact angle; filled box: specific activity.)

MCF–TBNCl and MCF–TMNCl have hydrophilic and amphiphilic surface properties, respectively. MCF–C8 and MCF–Ph have hydrophobic properties, which are in accordance with the results of contact angle measurement. The weight ratio of crude PCL/MCF was 0.5.
the order of MCF > MCF−TMNCl > MCF−C8 > MCF−Ph. However, \( \frac{d}{c} \) for all of the MCFs is similar, which indicates that interactions between the hydrophobic/hydrophilic surface of MCFs and benzene are comparable.

Crude PCL without purification was used in our work to minimize the prize of the final hybrid catalyst and farthest maintain the original activity of protein. Moreover, the presence of additive dextrin can help to stabilize the lipase confined into MCFs and maintain the natural structure of lipase. In the adsorption process, a low weight ratio of PCL/MCF was used to normalize the loaded amounts of PCL in various MCFs. Fortunately, when the weight ratio of crude PCL/MCF−R was 1, above 96% of PCL were successfully immobilized into functionalized MCFs, while unmodified MCF exhibited a lower adsorbing efficiency. As can be seen in Table 1, functionalized MCFs exhibit similar lipase loading (approximately 20 mg/g, corresponding to 13 000 units). However, the nature of the support surface can obviously affect the loaded amount of protein. Most of the functionalized MCFs did not reach saturated adsorption when the weight ratio of crude PCL/MCF−R was 1. If we further increase the weight ratio of PCL/MCF−R to 5, the lipase loading could be up to 100 mg/g in hydrophobic MCF−C8 and MCF−Ph. For unmodified MCF, the weight ratio of PCL/MCF was reduced to 0.5 and 96% of adsorbing efficiency was just achieved. The lipase loading of unmodified MCF is around 9.6 mg/g.

### 3.2. Influence of Surface Hydrophobicity/Hydrophilicity of MCFs on the Catalytic Performance of Immobilized PCL

A non-aqueous transesterification reaction of racemic 1-phenylethanol was selected as the model reaction to determine the catalytic activity of PCL. Crude PCL shows moderate performance with a specific activity of 145 \( \mu \text{mol min}^{-1} \text{g}^{-1} \) (Figure 3). When PCL was immobilized into unmodified MCF, the activity of PCL/MCF drastically decreases, probably because of the incompatibility of MCF and PCL. However, PCL immobilized into functionalized MCF exhibits enhanced specific activity compared to crude PCL, which indicates that confinement in a more hydrophobic microenvironment is beneficial for enhancement of the enzymatic activity of PCL in non-aqueous media. Obviously, the surface properties of MCFs affect the enzymatic activity of immobilized PCL. To investigate systematically the relationship between the catalytic performance of immobilized PCL and surface hydrophobicity/hydrophilicity of MCFs, specific activities of immobilized PCL were correlated with contact angles of MCFs. As shown in Figure 3, the specific activity of the immobilized PCL increases as the contact angle of MCFs increases, suggesting that the hydrophobic surface can increase the specific activity of immobilized PCL. PCL/MCF shows the lowest specific activity of 32 \( \mu \text{mol min}^{-1} \text{g}^{-1} \) at 25\( ^\circ \). Interestingly, a small increase in surface hydrophobicity with a contact angle from 25.5\( ^\circ \) for MCF to 35.0\( ^\circ \) for MCF−NH\(_2\) results in a rather large increase in specific activity, approximately 6 times enhancement. The highest specific activity of 804 \( \mu \text{mol min}^{-1} \text{g}^{-1} \) is achieved by MCF−Ph−supported PCL, which is about 25 times higher than that of PCL/MCF. Although the PCL/MCF−C8 series have similar PCL content and pore size (Table 1), their specific activities are enhanced with increasing amounts of octyl groups on the surface, which is in parallel with the tendency of the contact angle of the MCF−C8 series. We can conclude that surface hydrophobicity is a key factor influencing the activity of the PCL/MCF−C8 series. PCL/MCF−TMNCl and PCL/MCF−TBNCl also exhibit the same trend. The above results demonstrate that hydrophobic surfaces play the same role as the oil−water interface in activating PCL and lipase activation plays the key role in increasing the catalytic activity of PCL immobilized into hydrophobic MCFs.

Lipase activation via the local hydrophobic interaction between alkyl groups and the lipase molecule is very important for enhancing the catalytic activity of PCL. There are two different conformations in PCL, including the active form with an open lid and the inactive form with a closed lid. In aqueous media, these two conformations are in dynamic equilibrium. Many structural studies suggested that the “lid” and cleft around the active site are rich in hydrophobic residues. Alkyl groups in the MCFs could induce the “lid” open and further increase the accessibility of the lipid substrate to the active site. Therefore, the specific activity of immobilized PCL increases with the increase of the surface hydrophobicity of MCFs.

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**Table 2. Surface Hydrophobicity/Hydrophilicity of MCFs with and without Surface Modification**

<table>
<thead>
<tr>
<th>sample</th>
<th>molar ratio of adsorbed water/benzene (at ( P/P_0 = 0.9 ))</th>
<th>adsorbed water(^a)</th>
<th>adsorbed benzene(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \text{mL g}^{-1} )</td>
<td>( d_{\text{water}} ) (molecule nm(^{-2}))</td>
</tr>
<tr>
<td>MCF</td>
<td>1.28</td>
<td>141</td>
<td>8.65</td>
</tr>
<tr>
<td>MCF−TMNCl</td>
<td>0.67</td>
<td>118</td>
<td>10.36</td>
</tr>
<tr>
<td>MCF−C8 (0.69)</td>
<td>0.16</td>
<td>70</td>
<td>5.79</td>
</tr>
<tr>
<td>MCF−Ph</td>
<td>0.08</td>
<td>25</td>
<td>2.11</td>
</tr>
</tbody>
</table>

\(^a\)Monolayer adsorption capacity at 273 K.

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**Figure 4.** Comparison of hydrolitic activities of PCL immobilized into MCFs with various hydrophobic/hydrophilic surfaces. (Filled box: specific activity in tributyrin; unfilled box: specific activity in triacetin.)
The hydrolytic reaction of tributyrin and triacetin was further used to validate the “interfacial activation” of immobilized PCL. As shown in Figure 4, in the tributyrin system, the specific activity of immobilized PCL increases with the increase of the surface hydrophobicity of MCFs. Although there are obvious diffusion limitations of water in hydrophobic MCFs (see Table 2), PCL/MCF–Ph still exhibits the highest specific activity (15 984 μmol min⁻¹ g⁻¹) among the immobilized PCL series, indicating that PCL immobilized into hydrophobic MCFs is activated. However, in comparison to crude PCL (13 000 μmol min⁻¹ g⁻¹), most of the immobilized PCL exhibit decreased specific activity, which indicates that confinement of functionalized MCFs plays a negative role in the hydrolytic activity of immobilized PCL.

In the triacetin system, the specific activity of immobilized PCL increases as the contact angle of the MCF series increases and reaches a maximum at PCL/MCF–TMNCl, because the diffusion limitations of water and partly soluble triacetin drastically reduce the catalytic activity of PCL immobilized into hydrophobic MCFs and counteract the effect of hydrophobic activation. PCL/MCF–C8 and PCL/MCF–Ph still exhibit comparable specific activity to PCL/MCF–TBNCl, which also testifies the presence of hydrophobic activation.

3.3. Influence of Surface Hydrophobicity/Hydrophilicity of MCFs on the Catalytic Performance of Immobilized CALB.

To further confirm the “interfacial activation” of PCL in hydrophobic MCFs, Candida antarctica lipase B (CALB) was also immobilized into the siliceous MCFs with various hydrophobic/hydrophilic surfaces to catalyze the transesterification reaction of racemic 1-phenylethanol. CALB is a good replacement for PCL because it has a similar Ser-His-Asp catalytic triad to PCL, as well as comparable speci$c cation reaction of racemic 1-phenylethanol. However, CALB does not express “interfacial activation” because the putative “lid” (helix αS) above the active site cleft is so short and disordered that it cannot cover the active site, which is different from PCL.

As shown in Figure 5, CALB/MCF and CALB/MCF–TBNCl show similar specific activities (S16 and S27 μmol min⁻¹ g⁻¹, respectively). CALB/MCF–NH₂ and CALB/MCF–C8 (0.69) exhibit lower catalytic activity than CALB/MCF, whereas CALB/MCF–Ph shows slightly higher activity than CALB/MCF. Obviously, the specific activity of immobilized CALB does not strongly depend upon the surface hydrophobicity of MCFs. In comparison to crude CALB (280 μmol min⁻¹ g⁻¹), CALB immobilized into MCFs exhibit appreciably enhanced specific activity, which also indicates the presence of the host confinement effect. The above results further confirm that “interfacial activation” is the main reason for the higher specific activity of PCL immobilized into MCFs with a hydrophobic surface.

3.4. Relationship between Surface Hydrophobicity/ Hydrophilicity of MCFs and Intrinsic Fluorescence of Immobilized PCL.

The question of whether the higher specific activity of PCL immobilized into hydrophobic MCFs arises from “interfacial activation” promotes us to investigate the influence of surface hydrophobicity/hydrophilicity of MCFs on the structure of immobilized PCL. Fluorescence spectroscopy has been proven to be a powerful method to study the conformational change of protein. Especially, Trp(s) could be used as intrinsic probes because of the sensitivity of Trp fluorescence to the location and interactions within the protein. Of the 320 amino acid residues in PCL, three are tryptophan (Trp) residues. Trp284 is located near the Ser87-His286-Asp264 catalytic triad, and Trp209 is located close to the active site cleft, indicating that both Trp209 and Trp284 are buried in the interior of the protein surrounding the active site. Trp30 is located in the helix α1, which is close to the surface but points to

Figure 5. Comparison of specific activities of CALB immobilized into MCFs with various hydrophobic/hydrophilic surfaces for the non-aqueous transesterification reaction of racemic 1-phenylethanol.

Figure 6. Steady-state fluorescence spectra and time-resolved fluorescence spectra of crude PCL suspended in phosphate buffer and n-hexane.
the interior of the protein. The maximum emission wavelength $\lambda_{\text{max}}$ for Trp depends upon its microenvironment. Generally, for Trp in aqueous phase, $\lambda_{\text{max}}$ is 350–353 nm and a low-polarity and hydrophobic microenvironment causes a blue shift of $\lambda_{\text{max}}$. Steady-state fluorescence spectra of PCL suspended in buffer and n-hexane were depicted in Figure 6A. For PCL in phosphate buffer solution, $\lambda_{\text{max}}$ is 327 nm, suggesting that all of the Trp(s) within PCL are located in a more hydrophobic environment. When solvent was changed to n-hexane, the maximum emission $\lambda_{\text{max}}$ approximates that in buffer, indicating that Trp(s) are well-protected by the hydrophobic amino acid residue and not influenced by n-hexane. This is consistent with the fact that Trp(s) are buried in the hydrophobic interior of PCL and hardly accessible to water and n-hexane. Time-resolved fluorescence spectra of PCL are shown in Figure 6B. Curve a represents the fluorescence decay of PCL in buffer solution. Its intensity decay is approximately one-exponential, and the mean lifetime is 5.18 ns. Curve b represents the fluorescence decay of PCL suspended in n-hexane, and its intensity decay is three-exponential, with a mean lifetime of 3.73 ns. Because PCL molecules suspended in n-hexane are in the compact and aggregated state, the decreased lifetime probably originated from the quenching by the protein matrix.

Furthermore, we used time-resolved fluorescence spectroscopy to investigate the effect of surface hydrophobicity/hydrophilicity of MCFs on the structural change of immobilized PCL. PCL/MCF was used as the hydrophilic model. PCL/MCF–TMNCl was used as the amphiphilic model. Moreover, PCL/MCF–C8 and PCL/MCF–Ph were chosen as the hydrophobic models. The surface hydrophobicity/hydrophilicity of MCFs affects the fluorescence decay of immobilized PCL, as seen in Figure 7. In the phosphate buffer system, the fluorescence decay of PCL immobilized into hydrophobic MCFs becomes faster compared to that of PCL immobilized into hydrophilic MCFs (Figure 7A). However, the immobilized PCL exhibits the opposite trend in n-hexane (Figure 7B). Fitted results of time-resolved fluorescence spectra as a sum of three-exponential terms are shown in Figures 8 and 9. In the phosphate buffer system, the short-lived lifetime $\tau_1$ decreases from 0.6 to 0.1 ns and the long-lived lifetime $\tau_3$ shortens from 16.8 to 10.8 ns with the increase of surface hydrophobicity of MCFs, whereas the intermediate lifetime $\tau_2$ varies from 3.8 to 3.4 ns (Figure 8A). The mean lifetime ($\tau_{\text{mean}}$) of immobilized PCL decreases with the increase of the surface hydrophobicity of MCFs. Along with the decreasing fractional contribution of intermediate $\tau_2$ and long-lived $\tau_3$, the fractional contribution of short-lived $\tau_1$ increases from 19 to 79% (Figure 8B).
changes indicate that Trp(s) became exposed to a more polar microenvironment. These results lead us to propose the following activation model for the interaction between PCL and the support surface. When PCL is immobilized into hydrophobic MCFs, the hydrophobic interaction between alkyl groups and the so-called “lid” induces the “lid” to be opened, which provides a channel for water to enter into the active site cleft, resulting in an increase in quenching by water to Trp(s).6 When immobilized PCL was suspended in n-hexane, three lifetimes ($\tau_1$, $\tau_2$, and $\tau_3$) together with their mean lifetime ($\tau_{mean}$) increase with the increase of the surface hydrophobicity of MCFs (Figure 9A). This shows opposite trend compared to that of immobilized PCL suspended in phosphate buffer (Figure 8A). The fractional contribution of the short-lived $\tau_1$ increases from 31 to 41%, whereas the fractional contribution of the long-lived $\tau_3$ decreases from 28 to 17% (Figure 9B). The change of the fractional contribution of the intermediate $\tau_2$ is small. These results also support the above-mentioned PCL activation model. The hydrophobic support could avoid the aggregation of lipase molecules and induce the movement of “lid”, which permit n-hexane to enter into the active site cleft. Additional n-hexane decreases the quenching by the surrounding protein matrix, resulting in prolonged lifetimes.

4. CONCLUSION

In summary, PCL immobilized into MCFs with a hydrophobic surface exhibit much higher catalytic activity than PCL immobilized into MCFs with a hydrophilic surface. The enhancement of specific activities of immobilized PCL is ascribed to lipase activation originated from the hydrophobic interaction between alkyl groups and the “lid.” Time-resolved fluorescence spectra further confirm the conformational changes of PCL immobilized into MCFs with various hydrophobic/hydrophilic surfaces. When immobilized PCL was suspended in phosphate buffer, short-lived $\tau_1$ obviously shortened and the corresponding fractional contribution increased with the increase of the surface hydrophobicity of MCFs. In the n-hexane system, all of the lifetimes prolonged correspondingly. These results demonstrate that the hydrophobic surface induces a conformational change of immobilized PCL, so that the active site becomes accessible to the substrates and solvents. The results of the time-resolved fluorescence experiment together with the data of catalytic activity further confirm the enhancement of the catalytic activity of immobilized PCL by hydrophobic activation.

ASSOCIATED CONTENT

Supporting Information. Nitrogen sorption isotherms and corresponding pore size distributions (inset) of MCF series (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES
