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Comparison of the Properties of Lipase Immobilized onto Mesoporous Resins by Different Methods

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Abstract Genipin, a natural cross-linking agent, was used for the immobilization of lipase from *Candida* sp. 99-125 by cross-linking to two kinds of mesoporous resins. Under optimum conditions, the activity recovery of immobilized lipase on resin NKA-9 could reach up to 96.99% when the genipin concentration was 0.5%, and it could reach up to 86.18% for S-8 with a genipin concentration of 0.25%. Compared with using glutaraldehyde as a cross-linking agent, the immobilized lipase using genipin showed better pH and thermal stability, storage stability, and reusability. The residual activity of immobilized lipase using genipin as cross-linker remained more than 60% of its initial activity after six hydrolytic cycles, whereas only about 35% activity remained by using glutaraldehyde as cross-linker.

Keywords Immobilized lipase · Cross-linking · Genipin · Glutaraldehyde · Stability

Introduction

Lipase is an important enzyme in biological systems which usually participate in reactions such as hydrolysis, esterifications, and transesterifications. The advantages of lipase can be summarized as follows: mild conditions, high specificity, low-energy demand, and pollution-free [1]. Therefore, lipase has been widely used in pharmaceuticals, cosmetics, food and flavor making, feeds/feed additives, biodetergents, and biochemical industry such as oil and fat processing, leather production, paper manufacture, environmental pollution controlling, and chiral compound separation [2, 3].

Free lipase is water-soluble and often agglomerates in organic solvents that usually cause low catalytic efficiency, poor stability, difficulty of product recovery, and impossibility of

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reuse in industrial process [1]. In order to solve these disadvantages, lipase has been immobilized by a variety of methods including adsorption, covalent binding, cross-linking, and entrapment [4, 5]. Adsorption is the most popular method among these approaches due to its mild interactions, which can arouse lipase senior structural changes less and does not damage the active sites directly [5–7]. Due to the weak interactions between enzyme and support, the stability and reusability of the immobilized enzyme by adsorption are rather poor [8, 9]. Therefore, it is necessary to improve the stability of the immobilized enzyme.

The treatment of adsorbed enzyme on support with cross-linker can solve the above problems to a certain extent [9–13]. Glutaraldehyde is the most effective cross-linker [14–16], which can enhance the interactions between enzyme and support. However, glutaraldehyde usually causes damage to the active sites of the enzyme. Moehlenbrock et al. [17] suggested that glutaraldehyde was a harsher cross-linking agent and therefore might have more impacts on enzyme activity in comparison with dimethyl suberimidate during the cross-linking process of the mitochondria from the biofuel cell. Jones and Vasudevan [18] reported the cellulose hydrolysis catalyzed by carrier-free immobilized cellulose, and the results indicated that higher glutaraldehyde concentrations gave lower yields.

Hence, finding a less toxic cross-linking agent as alternative to glutaraldehyde is one of the strategies to improve the stability and activity of the adsorbed enzyme. Genipin is a naturally occurring iridoid compound extracted from gardenia fruits according to a modern microbiological process. As a water-soluble bi-functional cross-linking reagent, genipin reacts promptly with amines or proteins which have free amine groups such as lysine, hydroxylysine, and arginine [19, 20]. Recent studies show that genipin is an excellent natural cross-linker for proteins, gelatin, collagen, and chitosan cross-linking [21–24]. It was reported that genipin might replace glutaraldehyde with the advantages of stability and biocompatibility of the cross-linked products in biomedical applications, such as drug delivery [25], peripheral nerve regeneration [21], uniform cartilage regeneration [22], skin tissue engineering [23], and tissue fixation [24]. Additionally, genipin had been applied in leather processing [26], fabrication of food dyes [27], and in herbal medicine [28]. These studies strongly indicate that genipin has significantly lower cytotoxicity and higher biocompatibility compared with the commonly used glutaraldehyde.

Immobilizing biomolecules using genipin has been reported previously. Fujikawa et al. [29] cross-linked cytochrome C using genipin and analyzed the molecular weight of the cross-linked products by gel electrophoresis. The results suggested that genipin could cross-link cytochrome C intermolecularly to yield oligomerized cytochrome C. They also immobilized β-glucosidase in calcium alginate gel. The activity of the immobilized β-glucosidase with genipin was higher than using other cross-linking agents and retained 100% of the initial activity even after 12 repeated uses [30]. Then, glucoamylase, protease, and naringinase were also immobilized on chitosan beads by using genipin as cross-linker successfully. Fujikawa et al. published the patent entitled “Method of immobilizing enzymes on a support with iridoid aglycone cross-linking agents” [31]. However, there are a few reports available on the applications of genipin in lipase immobilization. To our knowledge, genipin as cross-linker for *Candida rugosa* lipase type VII immobilizing on chitosan beads had been investigated by Chiou and coworkers [32]. Therefore, using this natural biological cross-linking agent can still be attractive.

In this paper, we used mesoporous resins as supports and genipin as the cross-linking agent for lipase from *Candida* sp. 99-125 immobilization. The properties of the immobilized lipase, including the different methods of combining adsorption with cross-

linking, the optimum conditions of immobilization, pH and thermal stability, reusability, and storage stability, were studied systematically. The catalytic property was evaluated by the hydrolysis of olive oil.

Materials and Methods

Materials

Lipase from *Candida* sp. 99-125 (claimed activity 1,000 U/g) was obtained from Beijing CTA New Century Biotechnology Co., Ltd. (Beijing, China). Genipin (MW=226.23, 98% by HPLC) was purchased from Linchuan Zhixin Bio-Technology Co., Ltd. (Jiangxi Province, China). Mesoporous resins S-8 and NKA-9 (the parameters of resins are shown in Table S1) were provided by Tianjin Nankai Hecheng S&T Co., Ltd. (Tianjin, China). Olive oil of chemical grade (acid value ≤4.0) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and acquired from Kermel Chemical Reagent Co., Ltd. (Tianjin, China) and used as received without any further purification.

Preparation of Lipase Solution

Lipase from *Candida* sp. 99-125 in extraction powder was used as received. The crude lipase must be treated before using. Crude lipase powder (measured activity 19.20 U/mL) was dissolved in phosphate buffer solution (PBS, pH 7.5, 0.1 M) [7, 8] and centrifuged at 8,700 rpm, 4 °C for 15 min to remove insoluble components. The supernatant (measured activity 12.09 U/mL) was collected and prepared to be immobilized.

Preparation of Supports

Pretreatment of resins had been prepared. The resins were submerged into ethanol (95%, *v*/*v*) for 24 h, then rinsed with deionized water, subsequently treated with HCl (5%, *w*/*v*, 4 h) and NaOH (2%, *w*/*v*, 4 h), and rinsed with deionized water until neutral, then dried at 40 °C for 15 h. The treated supports were then stored in desiccator at room temperature [7]. The resins had been wetted with an appropriate amount of PBS (pH 7.5, 0.1 M) for 20 min prior to use and separated by removing the supernatant.

Preparation of Genipin Solution

Genipin, a white crystalline powder, was used as received and stored in a refrigerator at 4 °C. Genipin was dissolved in PBS at different pH values (from pH 3.0 to 10.0) because the cross-linking reactions by genipin were pH-dependent [20].

The Different Methods of Lipase Immobilization

A: Lipase solution (12.09 U/mL, 5 mL), genipin solution (0.5%, *w*/*v*, 5 mL, pH 7.0), and supports (S-8 or NKA-9, 200 mg) were added into the conical flask with plug (50 mL), subsequently reacted at 30 °C for 6 h in a shaking water bath at 150 rpm. The immobilized lipase was separated and rinsed several times with deionized water, then lyophilized under vacuum for 24 h, and stored at 4 °C until use.

B: After lipase solution (12.09 U/mL, 5 mL) and supports (S-8 or NKA-9, 200 mg) were shaken at 30 °C, 150 rpm for 4 h, genipin solution (0.5%, *w*/*v*, 5 mL, pH 7.0) was added to the system and reacted for 6 h. The next procedures were the same as described above.

C: The adsorption between lipase solution (12.09 U/mL, 5 mL) and supports (S-8 or NKA-9, 200 mg) was carried out at 30 °C, 150 rpm for 4 h. Genipin solution (0.5%, *w*/*v*, 5 mL, pH 7.0) was added to the particles which were separated and rinsed with deionized water and reacted for 6 h. The next procedures were the same as described above.

D: Supports (S-8 or NKA-9, 200 mg) were pre-activated by genipin solution (0.5%, *w*/*v*, 5 mL, pH 7.0) for 4 h. Then, lipase solution (12.09 U/mL, 5 mL) was added into the system at 30 °C, 150 rpm for 6 h. The next procedures were the same as described above.

E: Genipin (0.5%, *w*/*v*, 5 mL, pH 7.0) cross-linked lipase (12.09 U/mL, 5 mL) in a shaking water bath at 30 °C for 6 h, then reacted with supports (S-8 or NKA-9, 200 mg) at 30 °C, 150 rpm for 4 h. The next procedures were the same as described above.

Adsorption Efficiency Measurement

The supports after adsorption were separated by filtration and washed several times with a certain volume of deionized water, until no protein was detected in the washing solution. The supernatant and washing solutions were collected to determine the amount of protein by the Bradford assay [33], using our lipase solution as a standard.

The amount of leached lipase was determined by the amount of protein in the washing solution, and the amount of adsorbed lipase was calculated from the difference in the initial amount of protein in lipase solution and the total amount of protein in the washing solution and the supernatant solution after adsorption.

Adsorption efficiency (D) was determined by measuring the amount of protein in the solution before and after adsorption process, calculated by Eq. (1):

$$D(\%) = \frac{C_0 - C_\mathrm{i}}{C_0} \times 100\% \tag{1}$$

where C_0 denotes the initial amount of protein in lipase solution before adsorption (milligram), and C_i is the total amount of protein in the supernatant and washing solutions (milligram), namely, the amount that was not adsorbed.

Lipase Activity Determination

Lipase activity was determined according to the olive oil emulsion method [34]. The mixture of emulsion (4 mL) and lipase solution (5 mL, pH 8.0) was shaken in a water bath at 40 °C, 150 rpm for 30 min and stopped by the addition of acetone–ethanol solution (1:1, *v*/*v*, 10 mL). For immobilized lipase, immobilized lipase (200 mg) and PBS (5 mL, pH 8.0, 0.1 M) replaced the lipase solution, and the particles were separated from the mixture in order to stop the reaction. All experiments were done in triplicate and repeated three times, and the reported data were averages. The fatty acids released were determined by titration with 0.1 mol/L NaOH solution. One unit of lipase activity (U) was defined as the amount of lipase required to release 1 μmol fatty acid from olive oil per minute at 40 °C and pH 8.0.

Activity recovery (R) was calculated by Eqs. (2) and (3):

$$R(\%) = \frac{A_i}{A_f} \times 100\% \tag{2}$$

$$A_f = A_{f'} \times D \tag{3}$$

where A_i, A_f, $A_{f'}$, and D are the practical activity of immobilized lipase (unit), the theoretical activity of immobilized lipase (unit), the initial activity of free lipase used during immobilization (unit), and the adsorption efficiency, respectively.

Characterization Techniques

Specific surface area, total pore volume, and average pore diameter of immobilized lipase were measured by N_2 adsorption/desorption isotherms at 77.33 K using Micromeritics ASAP 2020. The pore size was calculated on the desorption branch of the isotherms using the Barrett–Joyner–Helenda method and the specific surface area was calculated using the Brunauer–Emmett–Teller method.

Results and Discussion

Effect of Different Methods on the Activity of Immobilized Lipase

Lipase from *Candida* sp. 99-125 was immobilized onto mesoporous resins S-8 and NKA-9 by different orders of adsorption and cross-linking. The results are shown in Fig. 1. The activity of lipase on S-8 resin was generally lower than NKA-9 because NKA-9 resin had weaker polarity and larger specific surface area which contribute to the adsorption of lipase [7]. The activity of immobilized lipase using method B was higher than others, which agreed with the results reported in literature earlier [9, 11]. This could be explained by the

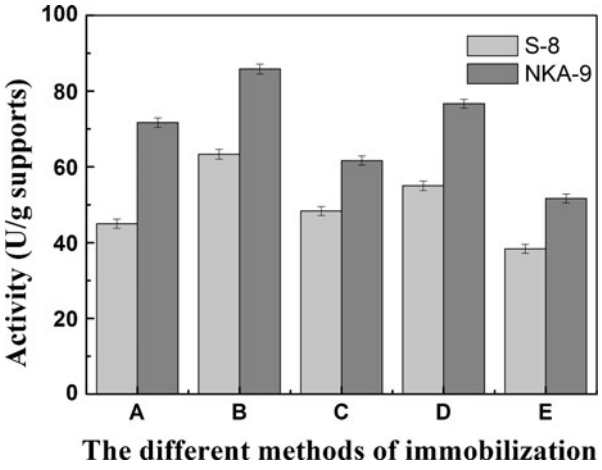

Fig. 1 Effect of different methods on the activity of immobilized lipase. The different methods were described in "Materials and Methods"

increase of lipase loading induced by the presence of cross-linker and lipase molecules during the cross-linking process. Methods A and D were similar for NKA-9 without amino groups. The cross-linking and adsorption that occurred at the same time could induce a lower loading of lipase using the two approaches. Resin S-8 was pre-activated by genipin in method D, and then lipase can be linked to the supports via genipin molecules [9]. In comparison with method B, the washing process during method C could increase the leaching of the adsorbed lipase. Additionally, the cross-linker would just reinforce the interactions of the adsorbed lipase molecules, but not bring new lipase molecules, while the lipase aggregates were formed before adsorption during method E and the diffusion of macromolecules was unfavorable. Based on the above results, the following experiments were conducted using method B.

Effect of Adsorption Procedure on the Activity of Immobilized Lipase

Method B is a two-step method which included adsorption and cross-linking. The operating conditions during the adsorption procedure could affect the final activity of immobilized lipase [6, 7]. Based on the single factor experiments (the data are not shown), lipase solution (12.09 U/mL, 5 mL) and supports (S-8 or NKA-9, 200 mg) that were shaken at 30 °C, 150 rpm for 4 h were been determined to be the optimum conditions of adsorption.

The adsorption efficiency of NKA-9 and S-8 could reach up to 53.57% and 28.34%, respectively. The activity recovery of the immobilized lipase on resin NKA-9 and S-8 could reach up to 98.67% and 90.33%, respectively.

Effect of Cross-Linking Time and Temperature on Activity Recovery of Immobilized Lipase

Cross-linking time affected the quality of immobilized lipase directly. As seen in Fig. 2, the highest activity was achieved when the cross-linking time was 6 h, which was considered to be the optimal condition. The longer cross-linking time gave the immobilized lipase a good

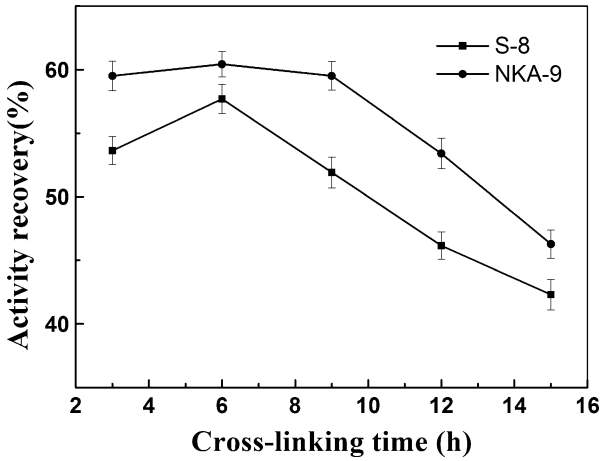

Fig. 2 Effect of the cross-linking time on the activity of immobilized lipase. Immobilization conditions: lipase solution (12.09 U/mL, 5 mL, pH 7.5, 0.1 M) and resins (200 mg) were adsorbed at 30 °C, 150 rpm for 4 h, then shaken gently at 20 °C for different times with the addition of genipin solution (0.5%, *w*/*v*, 5 mL, pH 7.0)

strength, while the cross-linking agent may have more impacts on the amino in the lipase active site that leads to more loss of activity [17, 18]. A similar behavior has been reported earlier [17].

From Fig. 3, it can be seen that the maximum activity recovery was obtained when the cross-linking process was at 20 °C, which was the optimal temperature for the cross-linking process, and the activity recovery decreased significantly with further rising of temperature. It was well known that higher temperature accelerated molecular movement and resulted in the reduction of lipase activity by strong interaction with genipin. Additionally, part of the lipase would be inactivated by thermal denaturation [7, 35].

Effect of Genipin Concentration and pH on Activity Recovery of Immobilized Lipase

Figure 4 shows that high genipin concentration results in low activity recovery. The optimal concentrations were 0.25% and 0.5% for S-8 and NKA-9, respectively, which were perhaps due to the characteristic of resins and the different amounts of lipase loading. The cross-linker might enhance the stability of the immobilized lipase, while the strong interactions could cause damage to the lipase since a high amount of cross-linker inactivates lipase [15]. A similar conclusion has been reported by Jones and Vasudevan [18]. Hence, optimization of the suitable cross-linker concentration was one of the most important factors to maintain the high activity of immobilized lipase.

The effect of the pH of genipin solution from 3.0 to 10.0 was observed, which was adjusted by the addition of HCl or NaOH solution to PBS. The results are shown in Fig. 5. The highest activity recovery was achieved at pH 6.0. During the experimental procedures, we found that the immobilized lipase showed a white appearance in the pH range of 3.0–4.0, and the color of the immobilized lipase changed gradually from pale blue to deep blue with the increase of pH value. Moreover, the genipin solution was faint yellow when the pH value reached 9.0–10.0, and the yellow color deepened with the passage of time. These indicated that genipin would undergo self-polymerization reaction prior to cross-link lipase with pH increased.

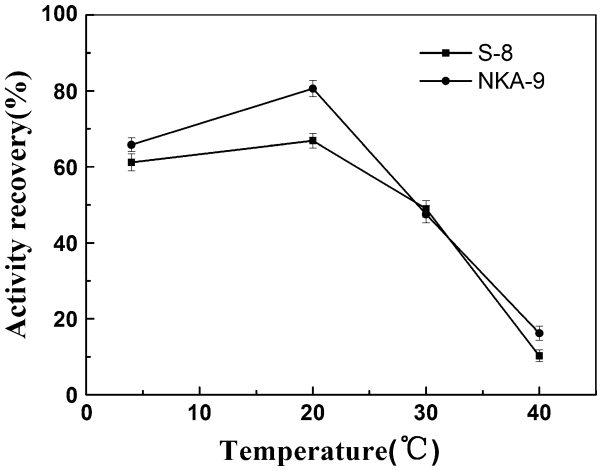

Fig. 3 Effect of the temperature for cross-linking on the activity of immobilized lipase. Immobilization conditions: lipase solution (12.09 U/mL, 5 mL, pH 7.5, 0.1 M) and resins (200 mg) were adsorbed at 30 °C, 150 rpm for 4 h, then shaken gently at different temperatures for 6 h with the addition of genipin solution (0.5% for NKA-9 and 0.25% for S-8, 5 mL, pH 7.0)

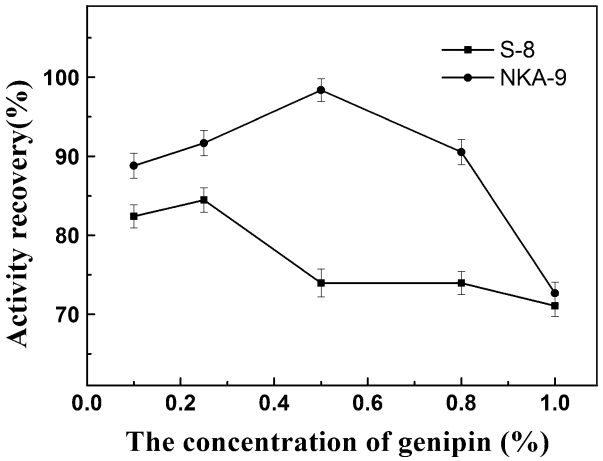

Fig. 4 Effect of the concentration of genipin on the activity of immobilized lipase. Immobilization conditions: lipase solution (12.09 U/mL, 5 mL, pH 7.5, 0.1 M) and resins (200 mg) were adsorbed at 30 °C, 150 rpm for 4 h, then shaken gently at 20 °C for 6 h. The genipin solution (5 mL, pH 7.0) with different concentrations from 0.1% to 1%

Similar behaviors that the cross-linking mechanisms of genipin were pH-dependent have been reported previously [36, 37]. Hence, pH 6.0 was selected for the following experiments to get an optimal compromise between activity recovery and cross-linking degree.

Cross-Linking with Glutaraldehyde

The lipase was cross-linked by glutaraldehyde with different concentrations (0.5%, 1%, 2%, and 5%, *v*/*v*) at the optimal immobilization conditions. The results are shown in Fig. 6. The higher concentration, the lower activity recovery, and the different activities at variable

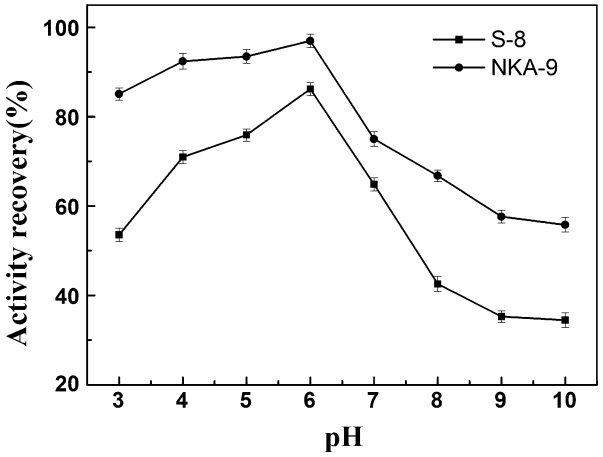

Fig. 5 Effect of the pH of genipin on the activity of immobilized lipase. Immobilization conditions: lipase solution (12.09 U/mL, 5 mL, pH 7.5, 0.1 M) and resins (200 mg) were adsorbed at 30 °C, 150 rpm for 4 h, then shaken gently at 20 °C for 6 h. The genipin solution (0.5% for NKA-9 and 0.25% for S-8, 5 mL) with different pH levels from 3.0 to 10.0

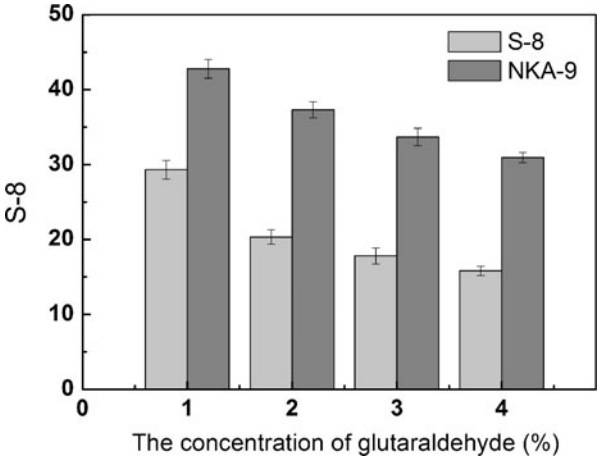

Fig. 6 Effect of glutaraldehyde on the activity of immobilized lipase. Immobilization conditions: lipase solution (12.09 U/mL, 5 mL, pH 7.5, 0.1 M) and resins (200 mg) were adsorbed at 30 °C, 150 rpm for 4 h, then shaken gently at 20 °C for 6 h. The glutaraldehyde solution (5 mL, pH 6.0) with different concentrations: 0.5%, 1%, 2%, and 5%

concentrations were explained previously. The activity recovery of lipase on S-8 was less than 30%, while the maximum activity recovery of lipase on NKA-9 reached 42.78%. In the present work, the final activity recovery of genipin cross-linked lipase on S-8 and NKA-9 achieved 86.18% and 96.99%, respectively. This suggested that the negative effects of glutaraldehyde on lipase were much greater than genipin.

pH and Thermal Stability of Immobilized Lipase

Each enzyme has an optimum pH. The ionization state of the enzyme active site could be affected by pH mild variation, and then the activity of the enzyme would be changed. Even many non-covalent bonds which used to maintain the three-dimensional structure of protein could be disrupted by the extreme pH, resulting in the denaturation of the enzyme [11, 12]. The 100% relative activity of lipase immobilized on resin S-8 and NKA-9 by cross-linking with genipin and glutaraldehyde was 71.20, 147.90, 22.70, and 64.70 U/g support, respectively, and 1056.17 U/g lipase for free lipase. The relative activity of free lipase maintained approximately 30% after incubation for 10 h at pH 3.0, while the relative activity of the immobilized lipase retained more than 80% by using genipin and around 60% for glutaraldehyde (as seen in Fig. S1). On the contrary, the performance in alkaline region was different. The activity recovery of immobilized lipase at pH 8.0 kept at 44.98%, 80.26%, 34.93%, and 64.7%, respectively, while the maximum activity of free lipase was reached. The shift of the optimum pH of lipase after immobilization was due to the uneven distribution of ions between the diffusion layer outside the immobilized lipase and the surrounding water phase caused by Donnan equilibrium in the immobilized lipase/water system [38]. Shifts in optimum pH with immobilization have been found for many enzymes [35, 39]. This indicated that the immobilized lipase was more suitable for catalysis reaction under acidic conditions. Moreover, the lipase immobilized on NKA-9 showed slight better stability than lipase on S-8.

The enzyme was sensitive to environmental changes, such as temperature. The thermal stability of immobilized lipase was determined and compared with free lipase. All of the

maximum activities were kept at 20 °C, which were 1098.11 U/g lipase, 63.33 U/g support, 155.0 U/g support, 22.17 U/g support, and 77.83 U/g support, respectively. The immobilized lipase with genipin showed unobvious advantage compared with free lipase; however, the resistance against high temperature was improved. This was because the cross-linking would make the conformation of lipase more rigid to prevent lipase molecular stretching deformation and molecular self-degradation [11]. Moreover, the excessive interaction could lead to counterproductive effects. The immobilized lipase on S-8 and NKA-9 by genipin maintained 32.14% and 16.98% of their relative activity after incubation at 60 °C for 2 h, respectively, while the free lipase kept 11.01% and the lipase cross-linked by glutaraldehyde lost its activity completely (the results are shown in Fig. S2). These results showed that the thermal stability of immobilized lipase with genipin was better than glutaraldehyde. One of the possible reasons was that the formation of bonding between lipase and supports and the cross-linking between lipase molecules by genipin were less harmful to the active sites of lipase. However, the essential reason on the mechanism was still not clear. Determining the mechanism of improving the thermal stability of immobilized lipase is a main objective in future research.

Reusability and Storage Stability of Immobilized Lipase

The immobilized lipase can be used repeatedly over an extended period of time. The immobilized lipase demonstrated a drop tendency in activity, which was ascribed to the following reasons: the leaching of lipase during repeated uses, the conformational changes, and lipase denatured [12]. The activity of lipase immobilized using genipin retained 61.33% and 65.62% after 6 cycles, respectively, whereas only about 35% activity of glutaraldehyde immobilized lipase remained (as seen in Fig. S3). The immobilized lipase using genipin was more stable than those using glutaraldehyde due to the lower cytotoxicity and the higher biocompatibility of genipin [21–25].

The storage stability of free and immobilized lipase was evaluated by determining the hydrolytic activity after storage at 4 °C for a period of time. The initial activity of lipase immobilized on S-8 and NKA-9 by cross-linking with genipin and glutaraldehyde was 77.18, 161.15, 27.33, and 73.17 U/g support, respectively; and the initial activity of free lipase was 1140.05 U/g lipase. The activity decreased with the extension of storage time, whereas the activity of lipase cross-linked by genipin decreased more slowly than glutaraldehyde. The free lipase maintained 72.33% of its initial activity after 25 days; however, the lipase immobilized on NKA-9 with genipin showed 57.99% residual activity, while 15.67% activity was preserved with glutaraldehyde (as seen in Fig. S4). Even though the storage stability of free lipase was better than immobilized lipase, the immobilized lipase could be used repeatedly during the industrial process. Additionally, the lipase immobilized by using genipin exhibited a significant storage stability than glutaraldehyde. This can be attributed to the low toxicity of genipin to the activity of enzyme during the storage period [19].

Characterization of Immobilized Lipase

The molecular weights of the lipase from *Candida* sp. 99-125 and genipin were 38 kDa and 226.23 Da, respectively. Lipase from *Candida antarctica* had a globular shape with approximate dimensions of 4.0 nm×4.0 nm×5.0 nm and relative mass 33 kDa [40]. So, the dimensions of lipase from *Candida* sp. 99-125 were similar as the dimensions of lipase from *C. antarctica* would be deduced. Hence, the pore size of the resins (15.5–16.5 nm for

NKA-9 and 28–30 nm for S-8) was possible to accept the lipase and genipin molecules theoretically. A similar result of adsorption of *C. antarctica* lipase B to porous polystyrene resin had been reported by Chen et al. [6].

Nitrogen adsorption/desorption isotherms and the pore size distribution of immobilized lipase on resins S-8 and NKA-9 are reported in Fig. S5 and Fig. S6. The average pore diameter of NKA-9 which loaded lipase and genipin decreased from 15.5–16.5 to 6.9 nm approximately, and from 28–30 to about 21.9 nm for S-8. A decrease in specific surface area was also observed: from 250–290 to 158 m^2/g for NKA-9 with immobilized lipase and from 100–120 to 95 m^2/g for S-8 with immobilized lipase. These results suggested that lipase and genipin molecules could be immobilized within the resin pores and not simply adsorbed on the external surface [41].

Conclusions

In this study, lipase from *Candida* sp. 99-125 was immobilized on two kinds of mesoporous resins by using a natural biological cross-linking agent: genipin. The enzymatic properties of the immobilized lipase were investigated and compared with those cross-linked by glutaraldehyde. The approach of cross-linking without tossing the residual lipase solution out following adsorption was adopted. Under optimum conditions, the activity recovery of immobilized lipase on resin S-8 and NKA-9 reached up to 86.18% and 96.99%, respectively. The immobilized lipase by using genipin showed better pH and thermal stability, storage stability, and reusability in comparison to glutaraldehyde, even though the performances in thermal stability and storage stability were not satisfactory enough. Conclusively, the results in this study strongly supported that glutaraldehyde could be replaced by genipin as an alternative cross-linking agent for enzyme immobilization. The success of lipase immobilized on resins by cross-linking with genipin expanded the application of genipin in biocatalysis.

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