Published in final edited form as:

Macromolecules. 2018 January 23; 51(2): 289–296. doi:10.1021/acs.macromol.7b02361.

Protection of opening lids: very high catalytic activity of lipase immobilized on core–shell nanoparticles

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Abstract

Various hydrophobic supports have been used for lipase immobilization since the active site of lipase can be opened in a hydrophobic environment. Nevertheless, the increase of lipase activity is still limited. This study demonstrates a hyperactivation-protection strategy of lipase after immobilization on poly(*n*-butyl acrylate)-polyaldehyde dextran (PBA-PAD) core-shell nanoparticles. The inner hydrophobic PBA domain helps to rearrange lipase conformation to a more active form after immobilization into the PAD shell. More importantly, the outer PAD shell with dense polysaccharide chains prevents the immobilized lipase from contact with outside aqueous medium and revert its conformation back to an inactive form. As a result, under optimal conditions the activity of lipase immobilized in PBA-PAD nanoparticles was enhanced 40 times over the free one, much higher than in any previous report. Furthermore, the immobilized lipase retained more than 80 % of its activity after 10 reaction cycles.

Graphical Abstract

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The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

FT-IR spectra of the polysaccharides, nanoparticles and the conjugation of nanoparticle and lipase, Fluorescence spectra of lipase, nanospheres and their conjugations, DLS data of PBA-PAD and PAA-PAD nanoparticles, the activity data of PAA-PAD-lipase.

Keywords

polyaldehyde dextran; nanoparticles; emulsion polymerization; lipase immobilization; interfacial activation

INTRODUCTION

Lipase is one of indispensable enzymes due to its key role in the metabolism of fats. It also catalyzes the synthesis and hydrolysis of a wide range of esters and amides.¹ Immobilization of lipase has played a critical role due to the ever-growing demand of stability and operability.^{2–8} The immobilization supports included inorganic materials such as nanoparticles^{9–11} or graphene oxides (GO),¹² organic materials such as fibers,^{13–14} gels,¹⁵ and polymers,^{16–17} as well as plentiful composites.^{18–20} The protocols for immobilization require surface modification and attachment usually influence the properties of lipase,^{21–22} and lead to the undesirable activity loss of lipase eventually.^{23–29} Additionally, lipase itself, due to unique molecular catalytic pathways, need to retain activity after immobilization. Most lipases, in contrast to other enzymes, catalyze the hydrolysis reaction at the water-oil interface.^{30–33} Active sites of many lipases are covered by an amphiphilic “lid”. In aqueous media, the “lid” usually closes. However, in organic solutions and more hydrophobic environment, it opens and has much higher activity.^{34–40} Therefore, many lipases are activated at the hydrophobic-hydrophilic interface. Recently, various hydrophobic supports such as octyldecyl-sepabeads and butyl-agarose have been used to immobilize lipase for this purpose.^{41–45} Octyl-glyoxyl agarose support was used to immobilize three different lipases by interfacial activation followed by covalent binding with 100 % retained enzyme activity.⁴⁶ Mesoporous organosilica with large cage-like pores was employed to immobilize lipase, resulting in three fold increased activity under optimal conditions.⁴⁷ Also, microcapsules formed via the assembly of enzyme-nanoparticle conjugation at the water-oil interface retained 76% of catalytic activity as compared with the free lipase.⁴⁸ Nevertheless, the existing efforts primarily focus on the activation of the catalytic site in lipase via immobilization onto hydrophobic surface by adsorption or covalent bonding. They cannot retain the open “lid” conformation. Therefore, the aim of this work is to efficiently preserve or even enhance the lipase activity by opening the lipase lid and protect the active conformation during immobilization.

In order to maintain the conformation of the activated lipase immobilized on the hydrophobic surface, we designed and synthesized amphiphilic core-shell nanoparticles with inner hydrophobic poly(*n*-butyl acrylate) (PBA) core and outer hydrophilic polyaldehyde dextran (PAD) shell via emulsion polymerization of *n*-butyl acrylate (*n*-BA) using PAD as an emulsifier and a macroinitiator^{49–53}. Porcine Pancreas Lipase (PPL) was chosen as a model lipase to be immobilized in the thin PAD shell of the nanoparticles by forming Schiff base linker between amino groups on lipase and aldehydes in the PAD shell under mild conditions (Scheme 1). The lipase, located in the shell, was activated by adjacent PBA core, which dramatically increased the lipase activity after immobilization. Meanwhile, the thin PAD shell offered a confined host space where lipase was protected from external aqueous

environment and retained its active conformation, benefiting from the biphasic interface of PBA-PAD and leading to surprising and prolonged catalytic performance.

EXPERIMENTAL SECTION

Materials

Dextran ($M_w = 40$ kDa) was obtained from Shanghai Ceneral-Reagent Titan Chem. Co., Ltd, China. Sodium periodate (NaIO_4 , 99 %, Aladdin, China) used for oxidation of dextran was kept in dark. *n*-Butyl acrylate (*n*-BA, 99 %, Aladdin, China) was stored at 4 °C and passed through an alumina column to remove the inhibitor prior to use. Lipase (EC 3.1.1.3, from porcine pancreatic, Sigma) and *p*-nitrophenyl palmitate (*p*-NPP, 98 %, Aladdin, China) were stored at −20 °C before use. Tris(hydroxymethyl) aminomethane (99.9 %, Aladdin, China) and Triton X-100 (99.9 %, Aladdin, China) are molecular biology levels. Hydroxylamine hydrochloride (98 %, Aladdin, China), potassium persulfate (KPS, 99%, Aladdin, China), ethylene glycol (99 %, Aladdin, China) poly(ethylene glycol) diacrylate (PEGDA, 99%, Aladdin, China) and other chemicals were used as received.

Synthesis of polyaldehyde dextran

Before the emulsion polymerization, dextran underwent partly oxidation treatment to obtain the aldehyde-functionalized emulsifier. Dextran (5 g, 30.9 mmol) was dissolved in 100 mL MilliQ water at 50 °C, followed by addition of NaIO_4 (6.61 g, 30.9 mmol) after cooling to room temperature. Ethylene glycol (1.92 g, 30.9 mmol) was added to terminate the oxidation after 2 h. The solution was dialyzed for 2 d and lyophilized to obtain white fluffy polyaldehyde dextran (PAD).

Determination of aldehyde concentration in PAD

The concentration of aldehyde (CA) was determined by titration, according to literature.⁵⁴ Briefly, PAD (>120 mg) was dissolved in 25 mL of hydroxylamine hydrochloride solution (0.25 M). The reaction was carried out at room temperature for 3 h. Then, several drops of methyl orange reagent (0.05 wt%) were added, followed by titration of standard sodium hydroxide solution (0.1 M) until the red-to-yellow end point. Five replicated experiments were performed each time. The aldehyde concentration was calculated as follows:

$$\text{CA (mmol/g)} = (V_1 - V_0) \times M/W, \quad (1)$$

where V_1 (mL) and V_0 (mL) are volumes of the standard sodium hydroxide solution and the blank control, respectively; M (mol L^{-1}) is the concentration of standard sodium hydroxide solution; W (g) is the weight of PAD.

Preparation of dense aldehydes-containing nanoparticles

High density aldehyde covered nanoparticles were prepared by emulsion polymerization in a one-step process. As an example, 0.2 g of PAD was first dissolved in MilliQ water for 0.5 h at 40 °C. 2 g of *n*-BA and 0.02 g of PEGDA were added after cooling the solution to room temperature. After deaerating with argon as well as pre-emulsification for 30 min, 0.01 g

KPS was added. The weight fraction of *n*-BA was maintained at 10 % of the oil-water mixture, the amount of KPS/*n*-BA was kept at 0.5 wt%. The fraction of PAD was varied between 1 wt% and 5 wt% to investigate the influence of the PAD concentration on the characteristics of final colloid particles such as monomer conversion, emulsifier conversion, size and its distribution. The polymerization was performed at 70 °C. The resulting latexes were de-emulsified by ethanol and centrifuged. Dry nanoparticles were obtained by washing them several times with distilled water and finally lyophilized. The conversion of *n*-BA (α) was calculated as follows:

$$\alpha_{n\text{-BA}} (\%) = (1 - c \times V/m) \times 100 \% \quad (2)$$

where *c* (mg/mL) is the concentration of *n*-BA in the supernatant determined by the absorbance at 235 nm; *V* (mL) is the volume of the supernatant; *m* (g) is the feeding weight of *n*-BA.

Surface properties of the nanoparticles

The overall content of PAD units chemically bonded to the PBA-PAD nanoparticles were determined by spectroscopy via anthrone method.⁵⁵ The density of aldehyde groups (ρ) on the particle after polymerization was calculated using a reaction with hydroxylamine hydrochloride, as mentioned above:

$$\rho (\text{mmol/g}) = w \times n \times \text{CA} / (0.1 + w) \quad (3)$$

where *w* (%) is the feeding weight percentage of PAD; *n* (%) is the PAD conversion; CA (mmol g⁻¹) is the concentration of aldehyde on PAD.

Immobilization of lipase to PBA-PAD nanoparticles

Immobilization of lipase on PBA-PAD nanoparticles was achieved by forming the corresponding imino (Schiff base) moiety under mild conditions. 1.0 g of nanoparticles was added into 1.0 mL of pH 7.4 phosphate buffered saline (PBS) containing divers lipase concentration (0.2 to 5 mg/mL) at 37 °C. After 5 min incubation and 60 min immobilization, nanoparticles were removed and washed by distilled water thrice and lyophilized.

Activity of lipase

Activities of both free and immobilized lipase were measured using 1 mM *p*-nitrophenyl palmitate (*p*-NPP) as substrate, dissolved in 50 mM Tris-HCl buffer solution (pH=8.0). The increase in absorbance at 405 nm caused by the release of *p*-nitrophenol in the hydrolysis of *p*-NPP was measured spectrophotometrically. 50 μ L of free lipase (or 150 mg immobilized lipase) was added to a mixture of 5 mL *p*-NPP solution and 5 mL of 0.01 M PBS (pH=7.4) and incubated for 5 min at 37 °C. The reaction was terminated by adding 10 mL of ethanol followed by centrifuging 10 min (10000 rpm). 2 mL of supernatant was mixed with the same volume of 1.0 M Na₂CO₃, and measured at 405 nm in a UV spectrophotometer. One unit (U) of enzyme activity was defined as the amount of enzyme which catalyzed the production

of 1 μmol *p*-nitrophenol per minute under the experimental conditions. Percentage of relative activity of enzyme was calculated as the ratio of specific activity of free or immobilized enzyme to the specific activity at their optimum conditions.

The optimal temperature for both free and immobilized lipase was studied at the range from 30 °C to 60 °C during 20 min. 50 μL of free lipase (or 150 mg immobilized lipase) was added to a mixture of 5 mL of *p*-NPP solution and 5 mL of 0.01 M PBS (pH = 7.4), which was incubated for 5 min at different temperatures. The optimal pH was investigated by changing pH value of PBS in the range of 3–11 for 20 min at 37 °C. The lipase activities under different conditions were measured as described above.

Thermal stability of immobilized lipase was studied at 4 °C, 25 °C and 45 °C. The immobilized lipase (free nanoparticles as control group) was placed at predefined temperature and taken out for activity measurement in a certain time interval. Similarly, pH stability of immobilized lipase was studied by varying the pH value in the range of 6 to 11. The immobilized systems and the control group were immersed in 0.01 M PBS for 24 h at 4 °C, followed by activity measurement at 37 °C (pH = 7.4) after incubation.

The reusability of the immobilized lipase was measured as follows: 150 mg of immobilized lipase (free nanoparticles as control) was added to 5 mL *p*-NPP solution and 5 mL of 0.01 M PBS (pH=7.4), incubated for 5 min at 37 °C and terminated by 10 mL of ethanol after 20 min reaction. The mixture was centrifuged for 10 min (8000 rpm). Concentrations of protein and *p*-NPP were detected in the supernatant. Then, nanoparticles with/without lipase were washed by PBS thrice, dried and above-mentioned experiments were repeated again for 10 cycles. Residual lipase content was calculated from the difference between the initial lipase amount loaded on the supports and that remaining in the aqueous solutions after centrifugation. Similarly, the relative residual lipase activity was calculated after reuse each time comparing to the initial activity of immobilized lipase.

Protein assay

Protein content was estimated by the method according to Bradford using the Bio-Rad protein dye reagent concentrate.⁵⁶ Bovine serum albumin was used as the standard.

Characterization

Fourier transform infrared (FT-IR) spectra were recorded using a PE Paragon 1000 spectrometer (KBr disk). The hydrodynamic diameter and size distribution of nanoparticles before and after immobilization were determined by dynamic light scattering (DLS) at 90° to the incident beam at 25 °C on a Brookhaven90 Plus particle size analyzer. All nanoparticles solutions had a final polymer concentration of 0.5 mg/mL and were filtered through a 0.45 μm filter. Transmission electron microscopy (TEM) images were conducted on a HT7700 electron microscope operating at an acceleration voltage of 100 kV. The samples for TEM measurements were prepared by placing a drop of 0.5 mg mL⁻¹ emulsion on the surface of Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. No staining was required. All the ultraviolet spectrophotometric (UV) measurements were performed on an ultraviolet-visible spectrophotometer (UV-2550, Shimadzu Ltd., Japan).

RESULTS AND DISCUSSION

Preparation of aldehyde-tailored dextran

PAD was prepared by partial oxidation of dextran using NaIO₄ according to the literature.⁵⁴ FT-IR spectra (Figure S1) show a fraction of hydroxyl groups was oxidized without any side reactions. The resulting aldehyde concentration in PAD was calculated by the reaction with hydroxylamine hydrochloride followed by titration with 0.1 M sodium hydroxide standard solution. A moderate oxidation of 8.36 mmol aldehydes g⁻¹ PAD was determined.

Synthesis of dense aldehydes-containing PBA-PAD nanoparticles

Acrolein and glutaraldehyde^{39, 57–58} are commonly used during the preparation or tailoring reactions of nanoparticles due to their facile reaction with biomolecules containing amines. Unfortunately, the hyper toxicity prevents their biomedical applications. Since the amphiphilic polysaccharides are useful tools for the control of surface characteristics of nanoparticles,^{59–63} we used PAD to act in the dual role of the emulsifier and macroinitiator for the emulsion polymerization of *n*-BA. The polymerization was performed at weight fraction of PAD varying from 1 wt% to 5 wt% with KPS as an initiator at 70 °C, to determine an optimal PAD feed concentration of the final nanoparticles. Stable latexes without coagulation were obtained in all cases. The size and morphology of PBA-PAD nanoparticles was studied by DLS and TEM. As summarized in Table 1, average size of nanoparticles decreased from 310 nm to 162 nm, as PAD feed concentration increased from 1 wt% to 5 wt%, as expected for emulsion polymerization. Additionally, quite uniform and stable nanoparticles with very narrow size distributions (< 0.02) were formed in all polymerizations. Figure 1 A–E shows the formation of spherically shaped monodisperse nanoparticles in water. Such a direct and efficient control of the final particle size by varying the PAD concentration can be potentially extended to the synthesis of other well-defined nanoparticles with controlled surface properties. The stability of PBA-PAD nanoparticles and their size distribution was monitored at the room temperature by DLS or 3 months. As shown in Figures 1 G–H, the average diameter of PBA-PAD nanoparticles in water did not change even after 3 months, confirming the nanoparticles stability.

To ensure that the nanoparticles are promising candidates for lipase immobilization, the residual monomer content and density of aldehydes on the surface of nanoparticles were determined. The residual *n*-BA concentration was measured by the change in absorbance at 235 nm using a UV spectrophotometer. As shown in Table 1, almost complete conversion of *n*-BA was determined after 10 h polymerization. In contrast to acrolein/glutaraldehyde containing polymers, this one-step polymerization strategy is simpler and the products are more biocompatible because of the polysaccharide nature. Conventional surfactants were physically absorbed onto water-oil interface to balance surface tension.

We hypothesize that the macromolecular PAD serves as a reactive emulsifier that becomes chemically bonded onto the surface of PBA core. This critically important observation was confirmed via high speed centrifugation (10000 rpm) followed by spectroscopy using anthrone method.⁵⁵ The content of PAD bonded onto nanoparticles was indeed correlated

with its initial feed concentration. Indeed, the aldehyde concentration increased from 0.469 mmol g⁻¹ to 2.75 mmol g⁻¹ as the PAD concentration increased from 1 wt% to 5 wt%.

Immobilization of lipase into core-shell PBA-PAD nanoparticles

Since the emulsion polymerization resulted in high solid content and essentially complete monomer conversion, the nanoparticles could be used directly without tedious post-processing. The simple Schiff base reaction between aldehydes in the hydrophilic shell of nanoparticles and amine groups on the lipase was employed to prepare lipase immobilized nanoparticles. The size of nanoparticles after immobilization showed an increase by a few nanometers compared to their precursors with PAD feed concentration increasing from 1 wt % to 5 wt% (Figure 2A). PBA-PAD nanoparticles with highest aldehyde density and smallest diameter (Sample E, Table 1) were used in the following immobilization study.

Lipase solutions with varied concentrations (0.2 – 5 mg mL⁻¹) were mixed with PBA-PAD nanoparticles to evaluate the immobilization efficiency at 37 °C and pH=7.4. As displayed in Figure 2B, saturated lipase loading content of 404 µg g⁻¹ nanoparticles was obtained with a high lipase loading efficiency (97 %) at the lipase concentration of 1 mg mL⁻¹. Compared with the results reported by other groups,^{13, 26, 64} the relatively low loading capacity may be due to the macromolecular chain of PAD intertwined to form the thin and tight shell, decreasing the lipase anchoring efficiency. DLS and TEM measurements were carried out to provide a deeper insight into the immobilization process (Figure 2C). The lipase immobilized nanoparticles were no longer regular spheres but had a size distribution broader than their precursors. Immobilization was more difficult in the heterogeneous environment and the concentrated saline conditions needed for immobilization could also influence the morphology of nanoparticles.

To address the question whether the growth of size of nanoparticles was caused by covalent immobilization of lipase, immobilized nanoparticles and the related control group were mixed in aqueous solution with the fluorescein isothiocyanate (FITC) to label protein by forming a thiourea bond.⁶⁵ An increase of fluorescence intensity at 517 nm was detected (Figure S2) after conjugating FITC on lipase immobilized PBA-PAD nanoparticles, followed by dialysis for one week. This confirmed the efficient immobilization of lipase in PBA-PAD nanoparticles.

Lipase Activity

In order to optimize the immobilization conditions, the effects of temperature and pH on the activities of free and immobilized lipase (Figure 3AB) were investigated at different temperatures (ranging from 30 to 60 °C) and pH values (ranging from 3 to 11). The optimal conditions required elevated temperatures (from 40 °C to 45 °C) and alkaline (pH 8 to 9) conditions after immobilization. The increase of optimal temperature is a universal phenomenon since immobilization increases the stability of lipase at high temperature.^{66–69} The higher pH value after immobilization could mainly be ascribed to the Schiff base bonds stability between PAD and lipase.

Thermal stability (Figure 3C) of immobilized lipase was investigated at three temperatures: typical storage temperature (4 °C), room temperature (25 °C) and optimal temperature

(45 °C). The stability of immobilized lipase decreased at higher temperature. Moreover, the activity of lipase showed a relative rapid decrease during the first 8 hours. The effect of pH on stability of immobilized lipase was studied at pH 6 to 11 (Figure 3D). Higher relative activity (> 75 %) appeared at pH 8 to 10.

Specific activities of lipase under optimal conditions were studied, as shown in Table 2. Free lipase showed maximal activity of $5.84 \times 10^4 \text{ U g}^{-1}$ lipase (40 °C, pH = 8). After immobilization, it increased to $2.35 \times 10^6 \text{ U g}^{-1}$ lipase (45 °C, pH = 9). Previously, the highest activity increase of lipase immobilized on chitosan was reported as 2110%.⁶⁸ The activity increase of nearly 40 folds (3924 %) in this study is remarkable. The lipases were immobilized into the thin shell of PBA-PAD nanoparticles because of the abundant aldehyde groups in PAD. On one hand, the inner PBA core provides sufficient hydrophobic environment for the activation of similar hydrophobic activity sites in lipases to force the open “lid” conformation. On the other hand, the PAD-covering environment facilitates the dispersibility of nanoparticles and good water solubility of immobilized lipase. The lipases in the PAD shell could be well protected from the direct contact with aqueous environment to increase their stability. The lipases directly immobilized on hydrophobic supports via absorption or chemical bonding without further protection, display activity loss,⁵⁸ or only a small activity enhancement.^{70–72} However, lipases reported here can maintain their open “lid” conformation and realize a very high activity and stability increase.

To further confirm that the hydrophobic-hydrophilic interface plays an important role for the promotion of lipase activity, the inner PBA was hydrolyzed (Sample E, Table 1) to form poly(acrylic acid) (PAA), and transformed from a hydrophobic to hydrophilic environment (Figure S1). The resulting PAA-PAD nanoparticles were then used as supports for lipase immobilization.

The PAA-PAD nanoparticles had a smaller size than PBA-PAD nanoparticles, in the range of 147 nm to 162 nm, as measured by DLS and TEM in PBS, pH = 7 (Figure S3). The effect of pH was also studied (Figure S4). At pH > 7, the size increased due to ionization of acidic groups and stronger electrostatic repulsions.

Catalytic activity of immobilized lipase on PAA-PAD supports was studied, as shown in Table 2 and Figure S5 (45 °C, pH = 8). The maximal activity under optimal conditions was $6.77 \times 10^5 \text{ U g}^{-1}$ lipase with a small effect of lipase loaded capacity ($402 \mu\text{g g}^{-1}$ particles), still higher than that of free lipase. However, it presented only 30 % activity of the lipase immobilized on PBA-PAD supports. The result confirms the importance of hydrophobic-hydrophilic interface microenvironment to the lipase activity.

Kinetic Studies

To better understand the surprising enhancement of lipase activity, kinetics of hydrolysis was investigated using a series of initial concentration (0–100 μM) of *p*-nitrophenyl palmitate (*p*-NPP) as a substrate. V_{max} and K_{m} as the main kinetic parameters were calculated from the double reciprocal as shown in Table 3. The much higher V_{max} value of $8.94 \times 10^6 \text{ U g}^{-1}$ lipase and the lower K_{m} value (0.92) were calculated for immobilization of lipase on PBA-PAD nanoparticles, as compared with free lipase and lipase immobilized on PAA-PAD

nanoparticles. This reveals that the properly designed hydrophobic-hydrophilic core-shell nanostructure effectively promotes the activity of lipase after immobilization. Furthermore, the activation energy (E_a) for lipase activity was evaluated (Table 3). This suggests the enhancement of affinity between lipase and substrates after the immobilization of lipase on PBA-PAD nanoparticles, corresponding well with the assessment of V_{max} and K_m , as discussed above.

Reusability Study

Compared with free lipase, the activity, stability and reusability of immobilized enzyme have great increased. It is especially important for chemical catalysis in a large scale. Immobilization of enzymes onto solid supports can facilitate more efficient separation of biocatalysts from substrates or products, as well as their recycling. The stability of the immobilized lipase in this study was estimated in PBS at pH 7.4 and 37 °C for 10 repeated cycles. The immobilized lipase was separated from substrate solution by high speed centrifugation (15000 rpm) after 20 min of hydrolysis, then washed and dried for the next recycle. After 10 cycles, the residual lipase content on the nanoparticles and the residual activity were reduced to 80 % and 74.5 % of the original values (Figure 4). This confirms the good stability and reusability of the immobilized lipase. The loss of the enzymes from the supports should be the main reason for the decreased activity. Chemical immobilization of the enzyme via covalent bonding prevents enzyme desorption and protects enzyme from denaturation by constraining it in a limited space. It is more efficient than the physisorption.

CONCLUSIONS

In conclusion, core-shell nanoparticles with dense aldehyde moieties in the shell were prepared through soap-free emulsion polymerization of *n*-butyl acrylate in the presence of PAD serving both as macroinitiator and the source of aldehydes after mild oxidation. The PAD shell entraps lipase and immobilizes it through Schiff base reaction. The immobilized lipase can adjust its conformation to expose its active site for catalysis, which is induced by the presence of hydrophobic PBA core. The PAD shell with dense polysaccharide chains helps to maintain the open conformation of lipase due to the restricted space and prevents the lipase from a contact with the aqueous environment. Overall, the PBA-PAD nanoparticles provide hyperactivation and stabilization of lipase, leading to very high activity (944 U g⁻¹ nanoparticles), which is nearly 40 folds higher than that of free lipase. It is the highest ever reported increase of lipase activity after immobilization. The core-shell structure of nano-size supports offers a new path to increase activity of immobilized lipase. The nanoparticles can be prepared in a large scale with essentially 100 % conversion of the monomers and polysaccharide. Therefore, we envision a potential for the large scale application of the new immobilization procedure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

WZ acknowledges supports from the National Natural Science Foundation of China (21674094), the Distinguished Young Investigator Fund of Zhejiang Province (LR18B040001) and the Fundamental Research Funds for the Central Universities (2017QNA4038). KM acknowledges a support from NIH (R01DE020843).

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Figure 1.

(A) to (E) TEM images of PBA-PAD nanoparticles at varying PAD feed concentration from 1% to 5%. (F) TEM image of PAA-PAD nanoparticles at 5 wt% PAD feed concentration. (G), (H) DLS traces for PBA-PAD nanoparticles.

Figure 2.

(A) Size of bare PBA-PAD nanoparticles and PBA-PAD-lipase conjugates at varying PAD feed concentration (1 – 5 wt%) by DLS. (B) Immobilization efficiency and lipase content as a function of lipase concentration (37°C, PBS, pH = 7.4). (C) Size and morphology measurements by DLS and TEM of bare PBA-PAD nanoparticles (upper, control group, under same PBS and incubation environment as immobilization except the lipase) and PBA-PAD-lipase conjugation.

Figure 3. Optimal temperature (A) and pH (B) of free and immobilized lipase in PBA-PAD nanoparticles. Thermal (C) and pH (D) stabilities of immobilized lipase in PBA-PAD nanoparticles.

Figure 4.
Reuse stability of lipase immobilized on PBA-PAD nanoparticles.

Scheme 1.
Preparation of dense aldehydes-covered PBA-PAD nanoparticles and lipase immobilization

Table 1

Characteristics of emulsion polymerization with various PAD concentrations^a

Sample	PAD feed content (wt%)	Size (d. nm) ^b	PDI ^b	<i>n</i> -BA Conv. (%)	PAD Conv. (%)	Aldehyde density (mmol g ⁻¹)
A	1	310.1	0.004	99.2	61.7	0.47
B	2	269.7	0.003	98.3	70.7	0.99
C	3	198.4	0.014	98.0	92.8	1.79
D	4	190.1	0.003	97.4	99.1	2.37
E	5	161.8	0.018	97.2	98.8	2.75

^a Polymerization conditions: 70°C, 10 wt% *n*-BA.

^b Measured by DLS.

Table 2
Activities and loading efficiency of free and immobilized lipase under optimal conditions

Types	T (°C)	pH	Activity (U g ⁻¹ lipase)	Activity (U g ⁻¹ particle)	Lipase loaded (μg g ⁻¹ particle)
Free lipase	40	8	5.84×10 ⁴	/	1000 ^a
PBA-PAD-lipase	45	9	2.35×10 ⁶	944	402
PAA-PAD-lipase	45	8	6.77×10 ⁵	282.3	417

^aConcentration of free lipase (μg mL⁻¹).

Table 3

Activation energy and kinetics parameters for free and immobilized lipase

Types	E_a (kcal g ⁻¹ mol ⁻¹)	$V_{\max}$ (U g ⁻¹ lipase)	K_m
Free lipase	3.13	6.88×10 ⁴	2.84
PBA-PAD-lipase	1.07	8.94×10 ⁶	0.92
PAA-PAD-lipase	2.57	8.05×10 ⁵	1.94