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Lipase Nanogel Catalyzed Transesterification in Anhydrous Dimethyl Sulfoxide

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The present work showed that *Candida rugosa* lipase, which is inactive in anhydrous dimethyl sulfoxide (DMSO), has been granted its original catalytic activity and greatly enhanced stability when encapsulated into a polyacrylamide nanogel. The molecular simulation and structural analysis suggested that the polyacrylamide nanogel shielded the extraction of essential water and maintained the native configuration of encapsulated lipase in anhydrous DMSO at an elevated temperature. The electron and fluorescence microscopy showed that the lipase nanogel would be well dispersed in anhydrous DMSO where its native counterpart aggregated. The encapsulated lipase behaved as a stable catalyst for transesterification between dextran and vinyl decanoate in anhydrous DMSO at 60 °C for 240 h and yielded a dextran-based polymeric surfactant with regioselectivity toward the C-2 hydroxyl group in the glucopyranosyl unit of dextran. All these indicated a high potential of enzyme nanogel for nonaqueous biocatalysis.

Introduction

The chemo-, regio-, and stereoselectivity of enzymatic catalysis in organic phase are of interest for the synthesis of pharmaceuticals, fine chemicals, and functional polymers with well-defined molecular structures that underpin their required performance.[1−6] For example, lipase-catalyzed esterification in the organic phase is regioselective toward polyhydroxyl groups of sugars and generates a product of a well-defined molecular structure in comparison to the route of chemical synthesis.[7] Having established that a native enzyme, protease from *Bacillus subtilis*, is active in anhydrous DMF, Klibanov and his co-workers have predicted unprecedented possibilities for carrying out enzymatic catalysis in organic solvents.[8] However and unfortunately, very few enzymatic catalysis processes have been realized in anhydrous dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF), which is known as 'universal solvent' and particularly useful for dissolving high molecular weight substrates. In addition to stripping essential water molecules from the enzyme surface,[9,10] DMSO and DMF dissociate the tertiary structure of the enzyme, leading to enzyme unfolding and deactivation.[11−14] Recent years have witnessed the growing efforts in using genetic engineering approach to overcome above-mentioned problem. Chen and Arnold obtained a 256-fold increase in the subtilisin activity by a random mutagenesis in 60% DMF.[15] Wong and co-workers extended the half-life of subtilisin from 15 to 350 h in anhydrous DMF using site-directed mutagenesis.[16] More recently Kroutil and co-workers identified a strain from *Paracoccus pantotrophus*, which secretes a DMSO-tolerant (up to 50% DMSO) alcohol dehydrogenase.[17] Given all this progress, the enzymes that work in anhydrous DMSO or DMF remain far from adequate, considering the immense potential of enzymatic catalysis in anhydrous DMSO or DMF, such as the derivation of mono-, di-, trisaccharides, polysaccharides,[18−22] and cellulose,[23,24] peptide synthesis.[25,26]

Recent progress in incorporating enzymes into nanostructures,[27−32] such as the encapsulation of enzymes in mesoporous silica,[33] single enzyme nanoparticles,[34] single enzyme nanogels,[35−37] and enzyme in molecular hydrogels,[38−42] has provided alternative methods for enhancing enzyme stability and activity under harsh conditions. Liu and his co-workers have demonstrated that after encapsulation in a polymer nanogel by in situ polymerization from the enzyme surface, enzymes such as horseradish peroxidase, carbonic anhydrase, and lipase have all been granted an enhanced stability against high temperature and organic solvents.[35−37] It is concluded from above-mentioned practices that, using the knowledge and techniques from enzyme chemistry, material chemistry, and computational chemistry,[37] it is possible to establish a suitable microenvironment for a chosen enzyme to catalyze organic synthesis in anhydrous DMSO and DMF.

The present study aimed to develop a comprehensive understanding of enzyme nanogel in anhydrous DMSO, a universal solvent for organic synthesis but strong denaturant for enzymes. Lipase, which is rapidly denatured in anhydrous DMSO,[43] was chosen as the model enzyme for fabrication of the enzyme nanogel and to catalyze the transesterification between dextran and vinyl decanoate in DMSO. This reaction is expected to yield a biocompatible and biodegradable polymeric surfactant with potential applications in cosmetics, food, and construction of polymeric micelles for drug delivery.[44] The enzyme stability, structural properties, and catalytic performance of the lipase nanogel in anhydrous DMSO were investigated using molecular dynamics simulation, structural characterization, and activity assays. To examine its feasibility for biocatalysis in DMSO, the lipase nanogel was applied to the transesterification between dextran and vinyl decanoate in anhydrous DMSO at 60 °C for 10 days.

Experimental Section

Materials. Lipase from *Candida rugosa* (Type VII, L-1754), *p*-nitrophenyl palmitate (*p*-NPP), acrylamide (AM), *N*-acryloxysuccinimide (NAS), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylene-diamine (TEMED), vinyl decanoate (VD), methanol, ethanol, DMSO, DMF, and fluorescein isothiocyanatepyrene (FITC) were purchased from Sigma-Aldrich, Dextran T-40 (from *Leuconostoc mesenteriodes*) with average molecular weight (Mn) 26000 g/mol and

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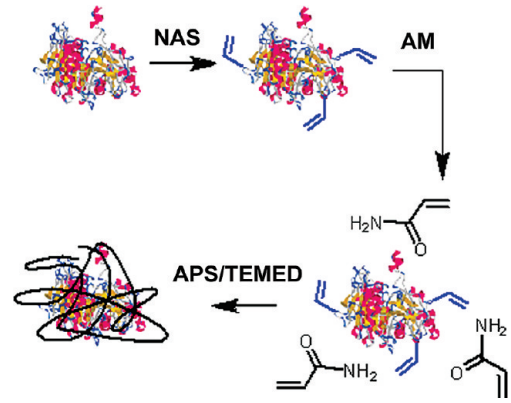
M_w 40000 g/mol according to size exclusion chromatography was bought from GE Healthcare. DMSO and DMF were dried over 3 Å molecular sieves overnight before use. Other chemicals were of analytical grade and used without further purification.

Synthesis of Lipase Nanogel. Lipase powder (4.0 g) was added to 200 mL of acetic buffer (50 mM, pH 4.0). This was followed by 10 min centrifugation at 10000 rpm and 4 °C to remove precipitates. NAS (355.8 mg), dissolved in 5 mL of DMSO, was added to the lipase solution (200 mL) dropwise in 10 min and the reaction lasted for 5 h at 30 °C, with stirring at 300 rpm. The modified lipase solution was then adjusted to pH 6.0 with 1 M NaOH before the addition of 10 g acrylamide powder. After 30 min N_2 purging, the polymerization was initiated by adding 400 mg APS and 500 μL TEMED and conducted under N_2 purging at 30 °C for 12 h. The product solution was then subjected to 24 h dialysis against water at 4 °C to remove unreacted reagents, followed by a 48 h lyophilization to obtain lipase nanogel in powder form. Size exclusion chromatography was conducted using a TSK-GEL SW4000xL column (TOSHO) with fluorescence detector (RF-10AxL, SHIMADZU) to determine the content of the encapsulated lipase. The modifying degree of the primary amine groups of lipase was determined by the fluorometric assay developed by Hoffman and his colleagues.[45] The encapsulation yield was determined by size exclusion chromatography with florescence detection and measured in terms of the peak area of the encapsulated lipase over the total area of protein fractions.

Assays. The hydrolytic activity of the lipase nanogel was determined using *p*-NPP as substrate.[46] For transmission electron microscopy (TEM), a drop of lipase nanogel aqueous solution (0.1 mg/mL protein content) was first placed on the carbon-coated grid. After removing the excess and applying 2%, pH 7.0 sodium phosphotungstate, the sample was visualized under TEM. For scanning electron microscopy (SEM), a drop of lipase nanogel (0.1 mg/mL protein content) was placed on the glass surface, dried at room temperature and directly visualized under SEM. To observe the microstructure of the lipase nanogel treated with DMSO, the samples were first dried from aqueous on the support, and then immersed in DMSO for 10 min. After removing excess, the samples were dried at room temperature for 10 days before visualizing under TEM and SEM. For fluorescence microscopy observation, native lipase or lipase nanogel (protein content 3 mg/mL) in 100 mM, pH 9.5 Na_2CO_3/$NaHCO_3$ buffer was reacted with FITC (0.2 mg/mL) at room temperature in the dark for 6 h; this was followed by dialysis against 50 mM, pH 7.0 phosphate buffer. Then a drop of the mixed solution of FITC-lipase and free polyacrylamide or a drop of FITC-lipase nanogel was dried on the surface of glass in the dark. The gel films were then examined using fluorescence microscopy.

Molecular Simulation. The structure of native *Candida rugosa* lipase was obtained from the Brookhaven Protein Data Bank (PDB code: 1TRH). The GROMACS 3.3.1 package was used to perform molecular dynamics simulation. Simulations were performed using general triclinic cell geometry. A free lipase molecule or a single lipase nanogel with different gel layer thicknesses was put into the center of the simulation box sized 16.48 × 16.445 × 14.773 nm. A certain number of DMSO molecules were added and the density of the system was about 1100 g/L. The single lipase nanogels were obtained from our previous simulation of the assembly composed of lipase and acrylamide molecules.[37] The number of DMSO molecules and water molecules in the simulation box were 33957 and 2211, respectively, chosen according to the catalytic experiment in anhydrous DMSO where water content was around 1.5%. To examine the effects of DMSO on the structures of the native lipase and lipase nanogel, the coordinate of the acrylamide molecules was fixed, that is, the layer of acylamide was kept intact during simulation, while lipase, water, and DMSO molecules were allowed to move. All bonds were constrained by using the LINear Constraint Solver denoted by LINCS in GROMACS. Hydrogen bonds were determined based on cutoffs for the angle (30°) acceptor−donor-hydrogen and the distance hydrogen-acceptor (0.35 nm).

Scheme 1. Two-Step In Situ Aqueous Polymerization To Prepare Lipase Nanogel



Synthesis of Dextran-VD. First, lipase L-1754 or lipase nanogel was dissolved in 200 mL of pH 7.5, 20 mM phosphate buffer, stirred at room temperature for 2 h, and lyophilized for 48 h. The water content of the lyophilized lipase and lipase nanogel powder, as determined by moisture analyzer (Sartorius MA 30, Medtel, Australia), was 8−11%. The protein content in the lyophilized lipase and lipase nanogel was determined to be 10% and 3.5%, respectively, by the bicinchoninic acid method. Then, dextran T-40 (1.0 g) and VD (1.2 g) ([VD]/[glucopyranoside unit] = 1/1) were dissolved in 15 mL of DMSO. After 5 min incubation at 60 °C, 0.8 g of pH-imprinted lipase L-1754 or 2.4 g of pH-imprinted lipase nanogel (protein content 240 mg) was added into the above DMSO solution. The reaction temperature was maintained at 60 °C and samples were taken periodically during the reaction. After centrifugation at 10000 rpm for 10 min, the supernatant was collected and dialyzed first against an ethanol−water mixture (85:15, v/v) for 2 days and then against another ethanol−water mixture (60:40, v/v) for 2 days. Finally it was dialyzed against distilled water for 2 days. The dextran-VD product in powder form was obtained after 48 h lyophilization. ^{1}H, ^{13}C NMR and ^{1}H−^{1}H correlation spectroscopy (COSY) were recorded on a JEOL JNM-ECA600 600 MHz spectrometer. A spectrum was recorded using a Nicolet 560-IR Fourier transform infrared (FT-IR) spectrometer.

Results and Discussion

Synthesis of Lipase Nanogel. Lipase nanogel was prepared by a two-step in situ aqueous polymerization as shown in Scheme 1. In the first step, *Candida rugosa* lipase was reacted with NAS to generate vinyl groups on its surface. The second step, that is, the in situ polymerization, was conducted over 2 h at 30 °C and yielded lipase polyacrylamide nanogels of 40 nm in diameter on average. The hydrolytic activity of lipase nanogels as determined by hydrolysis of *p*-NPP was 85%. Using the assay methods described in the experimental session, the modification degree was determined as 52%, as that reported in our previous work,[37] while the encapsulation yield reached 90%. A full coverage of protein surface by the polyacrylamide network is requested to prevent the leakage of essential water from enzyme surface in organic solvent. The high encapsulation yield is attributed to the assembly of acrylamide monomers around the lipase surface due to the intermolecular hydrogen bonding between lipase and acrylamide, as detailed elsewhere.[37]

To examine the tolerance of lipase nanogel to polar and hydrophilic solvents at high temperature, lipase nanogel was incubated in anhydrous DMSO, DMF, methanol, and ethanol at 50 °C for 3 h. As shown in Figure 1, native lipase was fully deactivated in DMSO, DMF, methanol, and ethanol while lipase nanogel preserved 80%, 120%, 70%, and 78%, respectively, of

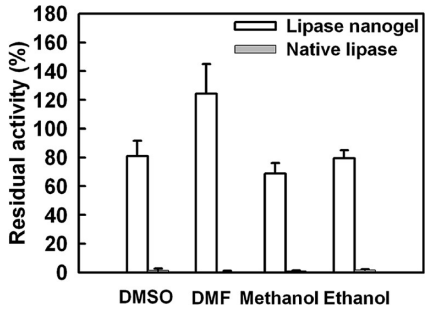


Figure 1. Comparison of stabilities of lipase nanogel and native lipase incubated in anhydrous polar solvents for 3 h at 50 °C.

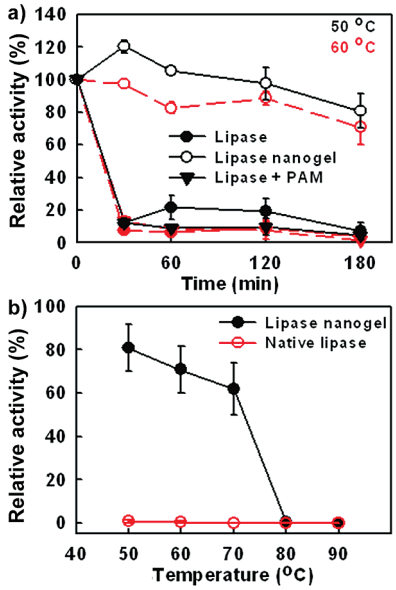


Figure 2. Comparison of stabilities of lipase nanogel and native lipase incubated in anhydrous DMSO at different temperatures.

its original activity. An increase in the lipase activity up to 20% has been observed after a short-term incubation of the lipase nanogel in either DMF (Figure 1) or DMSO (Figure 2), which, however, has not sustained. The enhancement of enzyme activity in nonaqueous phase has ever been reported by Klibanov and co-worker, in which they found that the presence of DMF and DMSO at a suitable concentration improved the conformational flexibility of enzyme and thus gave an enhanced activity.[47] While this could be included to the mechanism underlying the enhanced activity of lipase nanogel, a molecular insight into the interaction between the encapsulated lipase and the substrate in the presence of the denaturing solvent such as DMF and DMSO is needed to elucidate the catalytic behavior of the encapsulated lipase. In addition to the stripping of essential water from the enzyme, as observed in the case of polar solvents such as methanol,[37] DMSO and DMF can further dissolve the tertiary structure of enzyme, thus leading to a loss of enzyme activity.[11−14] The retention of the activity of lipase in anhydrous DMSO and DMF via chemical modification, to the authors' knowledge, has not previously been reported. Considering the similar deactivation mechanism of DMSO and DMF, DMSO was chosen for the following experimental and molecular simulation studies of the molecular details of the lipase nanogel.

Stability of Lipase Nanogel in DMSO. The stabilities of the lipase nanogel and native lipase were tested in anhydrous DMSO at 50 and 60 °C. As shown in Figure 2a, the native lipase lost most of its hydrolytic activity after 30 min, while the lipase nanogel preserved 80% of its activity within 180 min.

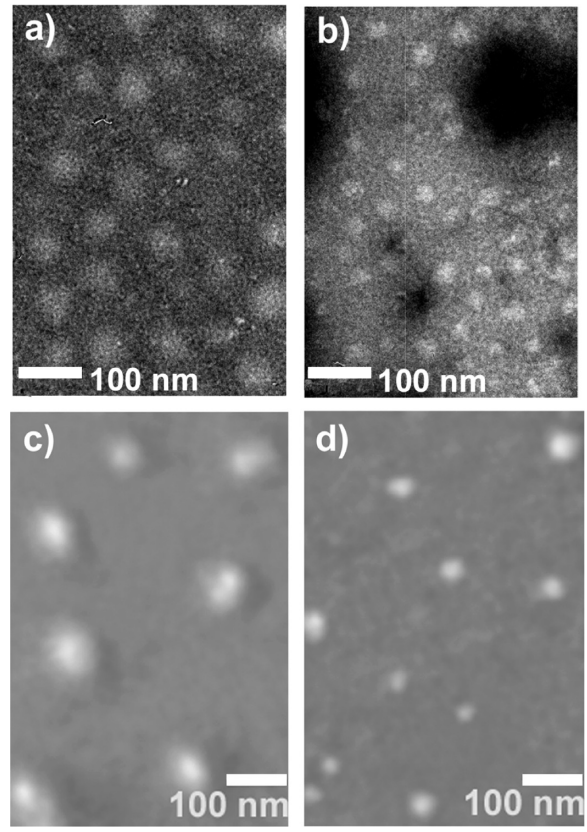


Figure 3. TEM of lipase nanogel: (a) dried in water, (b) treated with DMSO; SEM of lipase nanogel: (c) dried in water, (b) treated with DMSO.

As a control, polyacrylamide was added to the native lipase, and no enhancement was observed in enzyme stability. Furthermore, the free and the encapsulated lipase nanogel were both incubated in anhydrous DMSO at different temperatures for 3 h; their residual hydrolytic activities were then measured. As shown in Figure 2b, while native lipase demonstrated residual activity of below 1% at all measured temperatures, lipase nanogel exhibited 80, 73, and 62% of residual activity at 50, 60, and 70 °C, respectively. It was observed that the polyacrylamide gel became swollen in DMSO at 80 °C. This may lead to the failure of the gel network in stabilizing the tertiary structure of protein, the distortion of the encapsulated enzyme, and, consequently, the deactivation of the encapsulated lipase. A more concrete description of denaturing mechanism of the lipase nanogel at such a high temperature in DMSO requests complementary input from molecular simulation and structural characterization.

Characterization of Lipase Nanogel in DMSO. The TEM and SEM images shown in Figure 3a and c, respectively, suggest that the lipase nanogels obtained from the aqueous phase have an average diameter of 40 nm. When exposed to anhydrous DMSO, however, lipase nanogels shrunk to an average diameter of 13 nm, as shown by the TEM and SEM images in Figure 3b and d, respectively. This might be due to the extraction of the water molecules by DMSO and the consequential dehydration of polyacrylamide chains and intensified hydrophobic interaction of the main chains, leading to a phase transition of the gel network from the swollen to the collapsed state.[48−50] Considering the size of the shrunken state of lipase nanogel (~13 nm, Figure 3b and d) and that of the *Candida rugosa* lipase (7 nm, from the Protein Data Bank, PDB code: 1TRH), we arrived at the conclusion that each nanogel with the gel thickness of 3 nm contained only one lipase molecule inside. Moreover, Figure

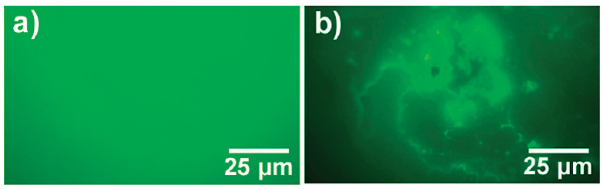


Figure 4. Fluorescence microscopy of (a) FITC-lipase nanogel film, and (b) FITC-lipase, polyacrylamide mixture film.

4 gives the spatial dispersion forms of FITC-labeled free lipase and lipase nanogel in the dried polyacrylamide gel films. Here the lipase nanogels are well dispersed (Figure 4a), in contrast to the aggregation of free lipase in the gel film (Figure 4b).

For conventional enzyme immobilization via either adsorption or encapsulation in a porous matrix, the significant mass transfer resistance in the matrix and the random distribution of enzymes on the matrix are the major reasons for the unsatisfactory catalytic efficiency. Here the encapsulation in a thin and porous gel presented an effective way to facilitate the transport of both substrate and product. Thus, the hydrolytic activity of lipase nanogel reached 85% of its native counterpart in free form. Similar results have also been reported for other enzymes.[35−37] The fluorescence microscopy analysis (Figure 4a) shows that lipase molecules are well dispersed in the dried gel matrix as immobilized form, in contrast to the aggregation of free lipase in the dried gel matrix (Figure 4b). This is also important in facilitating the full display of the catalytic power of each single enzyme in organic synthesis.

Protein Structure of Free Lipase and Lipase Nanogel in Anhydrous DMSO: Molecular Simulation. The conformations of free *Candida rugosa* lipase and its counterpart encapsulated in polyacrylamide gels of 3 nm thickness in anhydrous DMSO at 60 °C were simulated according to the method described in the molecular simulation section. As can be seen from Figure 5, after 3.5 ns in DMSO at 60 °C the tertiary structure of free lipase (Figure 5b) was distinctly different from that of its native conformation (Figure 5a), indicating that an unfolding occurred. In contrast, the encapsulated lipase (Figure 5d) maintained its initial conformation similar to the native one (Figure 5c). Moreover, the simulation also showed that the free lipase was

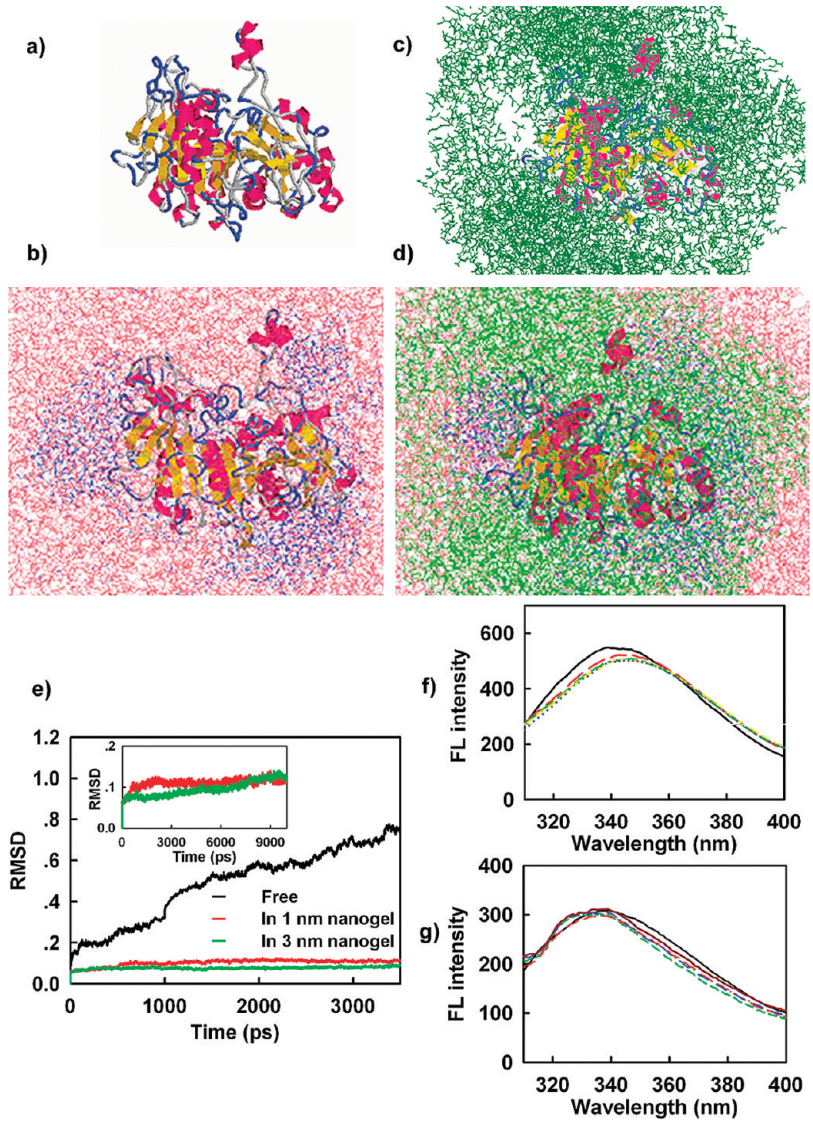


Figure 5. (a) Crystal structure of native lipase. (b) Structure of free lipase at 60 °C in DMSO. (c) Lipase encapsulated in nanogel. (d) Structure of lipase nanogel at 60 °C in DMSO (red, DMSO; blue, water; green, polyacrylamide shell). (e) RMSD of native lipase, lipase in nanogel with 1 and 3 nm gel layer at 60 °C in DMSO. (f) Fluorescence spectrum of native lipase in DMSO at 60 °C for 30, 60, 120, and 180 min. (g) Fluorescence spectrum of lipase nanogel in DMSO at 60 °C for 0, 30, 60, 120, and 180 min. (Native lipase and lipase nanogel were incubated in DMSO with protein concentrations of 0.5 mg/mL, followed by dilution in aqueous for fluorescence measurement.)

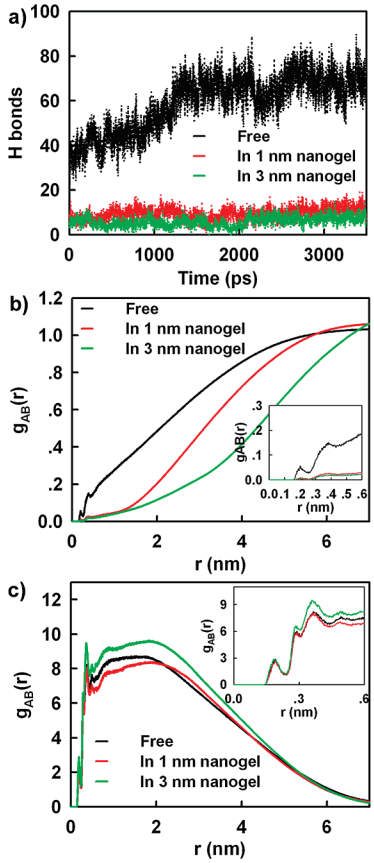


Figure 6. (a) Number of H-bonds between lipase and DMSO in case of free lipase and lipase in nanogel with 1 and 3 nm gel layers at 60 °C in DMSO. (b) The distribution of DMSO around lipase in the case of free lipase, and lipase in nanogel with 1 and 3 nm gel layers at 60 °C in DMSO. (c) The distribution of water around lipase in case of free lipase, and lipase in nanogel with 1 and 3 nm gel layers at 60 °C in DMSO.

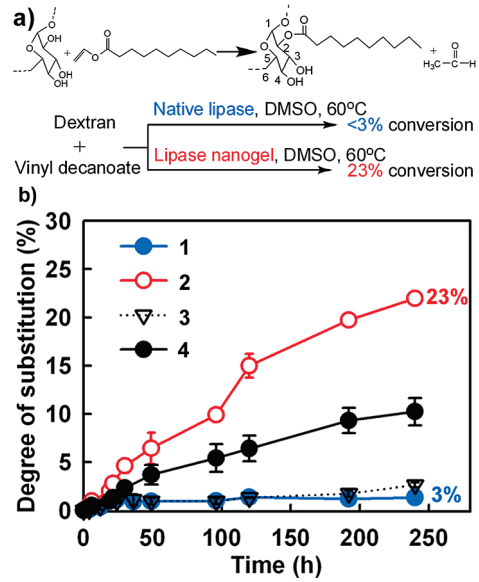


Figure 7. (a) Comparison of native lipase and lipase nanogel for organic synthesis in DMSO. (b) Conversion curves of transesterification between dextran and VD catalyzed by lipase and lipase nanogel: (1) native lipase, 60 °C; (2) lipase nanogel, 60 °C; (3) mixture of lipase and polyacrylamide, 60 °C; (4) lipase nanogel, 50 °C.

fully exposed to DMSO (pink), which penetrated into the interior region of lipase (Figure 5b), while the polyacrylamide layer (green) shielded DMSO from the lipase surface (Figure 5d).

The root-mean-square distance (RMSD) values are a statistical index of the conformational similarity. The RMSDs of the backbones of free lipase and the lipase nanogel in DMSO at 60 °C were monitored and are shown in Figure 5e. Here a continuous increase of RMSD from 0 to 0.75 in the first 3.5 ns is observed for free lipase, that is, a rapid unfolding of the lipase. In contrast, a minor increase to 0.12 is observed for the encapsulated lipase during 10 ns (Figure 5e, inserted). This confirms that the nanogel has preserved the tertiary conformation of the encapsulated lipase, as shown in Figure 5d. Figure 5f and g are the fluorescence spectra of native lipase and lipase nanogel incubated in anhydrous DMSO at 60 °C. A red-shift in the maximum emission wavelength (from 339 to 347 nm) of the native lipase appears after 30 min incubation, indicating a change in the tertiary structure of the lipase. In contrast, the fluorescence spectrum of lipase nanogel remains unchanged during the 180 min incubation, a stabilizing effect predicted by the molecular simulation shown in Figure 5e.

Hydrogen Bonding and Spatial Distribution of DMSO around Lipase: A Molecular Insight. The number of hydrogen bonds between DMSO and lipase in either free or encapsulated form was obtained from the molecular simulation described briefly in the experiment section and detailed in the Supporting Information. The numbers of hydrogen bonds formed between DMSO with the free and encapsulated lipase after 3.5 ns incubation in anhydrous DMSO at 60 °C are given in Figure 6a. The significant reduction of the number of H bonds formed between the lipase and DMSO due to the presence of a polyacrylamide gel indicates that the lipase is shielded from direct contact with DMSO. Consequently, as shown by the spatial solvent density profiles (Figure 6b), DMSO molecules are prohibited from penetrating the interior region of the lipase (Figure 6b insert). A thicker gel layer (3 nm) gives a further delayed increase in DMSO density around the lipase. This means that DMSO molecules are pushed further away from lipase, so there is a better shielding effect in a thicker gel layer. The spatial distribution of water molecules in the above-mentioned condition is given in Figure 6c. It is interesting to note that in the case of lipase in a polyacrylamide gel of 1 nm thickness, the water density profile is similar to that of free lipase as a reference. In other words, the gel layer of 1 nm is not adequate to overcome the stripping of essential water from the lipase surface by DMSO. Once the gel depth is increased to 3 nm, the water density is increased both in the vicinity of the encapsulated lipase, as shown by the third peak in Figure 6c (inserted), and in the surrounding region in the gel layer (Figure 6c). The enrichment of water in the vicinity of the encapsulated lipase and in the gel layer might be attributed to the formation of hydrogen bonds between the water and the polyacrylamide network around the lipase. This hindered the stripping water from lipase surface by DMSO and preserved the essential water for lipase to carry out its catalytic activity.

Synthesis of Dextran-VD by Lipase Nanogel in DMSO. To demonstrate the feasibility of lipase nanogel for organic synthesis in anhydrous DMSO, the synthesis of a hydrophobic dextran derivative from dextranT-40 and VD was carried out using native lipase and lipase nanogel, respectively (Figure 7a). In this reaction, *Candida rugosa* lipase L-1754 or lipase nanogel with the same protein content (240 mg) was added to 15 mL DMSO containing 1 g dextran T-40 and 1.2 g VD ([VD]/[glucopyranoside unit] = 1/1); this was followed by a reaction at 60 °C under vigorous stirring. After completion of the reaction, the degree of substitution of purified product was

determined from integrals of the ^{1}H NMR spectra (see Supporting Information).

As shown in Figure 7b, native lipase L-1754 from *Candida rugosa* gave a substitution degree of dextran lower than 3% after the reaction proceeded for 10 days. In comparison, lipase nanogel recorded a substitution degree of up to 23%. The stable and linear increase in the substitution degree indicates that the lipase nanogel retained its catalytic capability in anhydrous DMSO at 60 °C for the test period of 240 h.

The chemical structure of dextran-VD with a substitution degree of 23% was confirmed by FT-IR, ^{13}C NMR, and ^{1}H−^{1}H COSY (see Supporting Information). Using the method proposed by Yoshimoto et al.[51] and Therisod and Klibanov,[7] it was found that the modified glucopyranosyl residues were monosubstituted with a more favored regioisomer at the 2-position (ratio of 2- to 3-position was 7:3). This was consistent with the regioselective acylation of secondary hydroxyl groups in glucose by *Candida rugosa* lipase catalysis in pyridine, reported by Therisod and Klibanov,[7] with a 2- to 3-position ratio of 6:4.

Zaks and Klibanov have demonstrated that native lipase from *Candida rugosa* was completely inactive in DMSO.[43] Recently, Kaewprapan et al. applied native lipase L-1754 (*Candida rugosa* lipase) obtained from Sigma to catalyze the reaction between dextran and VD in DMSO and obtained a conversion of only 2.09% after 25 h at 50 °C,[52] while at the same time they found that native lipase AY (*Candida rugosa* lipase) from Amano, lipase from the same origin but a different supplier, showed activity in catalyzing the reaction between dextran and VD in DMSO, probably due to the different biochemical compositions of *Candida rugosa* lipase produced by different fermentation conditions. However, native lipase AY exhibited serious deactivation with a loss of 80% of initial activity within 1 h and a loss of almost all transesterification activity within 11 h, at 50 °C in DMSO.[52] In contrast, the lipase nanogel developed by present study essentially maintained its initial activity for transesterification in anhydrous DMSO at 60 °C within 240 h, as can be interpreted from Figure 7b. Lalonde et al.[53] have synthesized cross-linked enzyme crystals (CLECs) of *Candida rugosa* lipase via glutaraldehyde treatment, which retained 25% of activity after 10-day incubation in 50% DMSO at 25 °C. For the *Candida rugosa* lipase nanogel synthesized in the present study, as shown in Figure 7b, there appears to be no visible reduction in lipase activity during the 10 day reaction in anhydrous DMSO at 60 °C. This indicates the microenvironment formed by the hydrophilic polymer gel network contributes much more significantly to the stabilization in anhydrous DMSO, as compared to that by cross-linking of enzyme surface due to the presence of polyacrylamide. Despite the significantly improved stability of the encapsulated lipase as the catalyst, the conversion rate of dextran is modest. The improvement of the conversion rate might be realized by (1) increasing the content of Lip 3 isoenzyme that is specific for the esterification of cyclic alcohol in the commercial *Candida rugosa* lipase;[54] and (2) enhancing the mass transfer of the high molecular weight dextran as substrate to the encapsulated lipase.

Conclusions

The present work has shown the effect of encapsulating lipase from *Candida rugosa* in a polyacrylamide nanogel. After encapsulation in a nanogel, the lipase which would otherwise be denatured in anhydrous DMSO, a solvent that is conventionally considered as a denaturing reagent for proteins, maintained its biological activity, and behaved as a stable catalyst in anhydrous DMSO. The high stability and activity of lipase nanogel were further displayed in the synthesis of a hydrophobic derivated dextran performed in anhydrous DMSO at 60 °C for 10 days. The substitution degree of dextran reached 23% with a remarkable regioselectivity by discriminating among the three available secondary hydroxyl groups in the glucopyranoside unit. All these mark a noteworthy progress toward the ultimate dream of fully expanding the spectrum of nonaqueous enzyme catalysis. Recalling the enhancement in both thermal stability and tolerance to organic solvents that has been validated for horseradish peroxidase, carbonic anhydrase, and now in addition lipase, encapsulation has been shown to be a simple, effective, and universal technique. Development of this technique should rekindle interest in maintaining enzymatic activity in organic solvents, given the increased stability that has been demonstrated; it could greatly expand enzymatic catalysis in organic solvents, and inspire a broad exploration of chemical reengineering of biological systems for various applications.

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Supporting Information Available. NMR and FT-IR data and molecular simulation details are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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