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# Encapsulation of enzyme in metal ion-surfactant nanocomposites for catalysis in highly polar solvents

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We described a method to encapsulate enzyme in metal ion-surfactant nanocomposites. The nanobiocatalyst displayed highly retained activity (~100%) and much improved stability in protein-denaturing organic solvents. As a demonstration, the encapsulated lipase exhibited much higher activity and stability in the synthesis of vitamin E succinate in dimethyl sulfoxide.

Recently, there has been increased interest in research concerning the incorporation/encapsulation of enzymes/proteins in various metal ion-containing nanomaterials to produce nanoscale-immobilized enzymes and protein nanocomposites exhibiting excellent biological functions for applications involving industrial biocatalysis, biosensing, and protein delivery.[1–9] Metal ion-containing nanomaterials, such as inorganic nanocrystals and metal-organic frameworks, usually contain a significant number of metal ion sites on their surface. Enzyme can be conveniently attached to these surfaces via coordinated or electrostatic interactions between amino acids and metal ions under ambient conditions, often resulting in high degrees of enzyme loading and effective protection of protein configuration and enzyme activity.[1,3,4,8,10] Moreover, metal ions present in these nanomaterials can also serve as cofactors that enhance enzyme activity in some cases, allowing a much higher level of activity relative to that observed in the free enzyme in solution.[1,11] Additionally, enzyme incorporation can be easily achieved during synthesis of the metal ion-containing nanomaterials by a co-precipitation or biomimetic mineralization procedure.

These previous studies revealed that metal ion-containing nanomaterials represented new generation of nanocarriers for enzyme immobilization. However, most of these studies focused on the use of inorganic nanocrystals and metal-organic frameworks, which are relatively rigid inorganic nanomaterials that can increase the structural rigidity of the encapsulated enzyme. Although this can enhance enzyme stability,[12,13] it might also compromise the structural flexibility of the enzyme during catalysis, thereby potentially reducing catalytic activity.

In this study, we proposed using organic surfactants to replace the counter ions or ligands in the preparation of inorganic nanocrystals and metal-organic frameworks to construct metal ion-surfactant nanocomposites capable of simultaneously encapsulating enzymes. We hypothesized that encapsulation of an enzyme in the soft metal ion-surfactant nanocomposite might result in high levels of both enzyme activity and stability. Polydopamine (PDA)[14–16] was subsequently utilized to cross-link the enzyme-encapsulated metal ion-surfactant nanocomposites and facilitate the recycled use of the biocatalysts. The lipase-catalyzed synthesis of vitamin E succinate[17,18] in organic solvent was performed to demonstrate the high degrees of enzyme activity and stability displayed by the lipase-encapsulated metal ion-surfactant nanocomposite.

Preparation of the enzyme-encapsulated metal ion-surfactant nanocomposites and subsequent PDA cross-linking is schematically shown in Fig. 1. In a typical experiment, a solution containing 10 mM sodium deoxycholate (NaDC) and 0.18 mg/mL lipase from *Candida rugose* (CRL) was added to a water solution containing 20 mM cobalt chloride. After stirring for 30 min at room temperature, the mixture was centrifuged and washed with water twice to obtain the lipase-encapsulated metal ion-surfactant nanocomposites (CRL-MSNC). The CRL-MSNC was then incubated in freshly prepared 2 mg/mL dopamine solution in 10 mM Tris buffer (pH 8.0) for 24 h at room temperature to enable the self-polymerization of dopamine and cross-linking to CRL-MSNC (see the ESI† for experimental details). The Na content in CRL-MSNC and PDA@CRL-MSNC was determined by inductively coupled plasma

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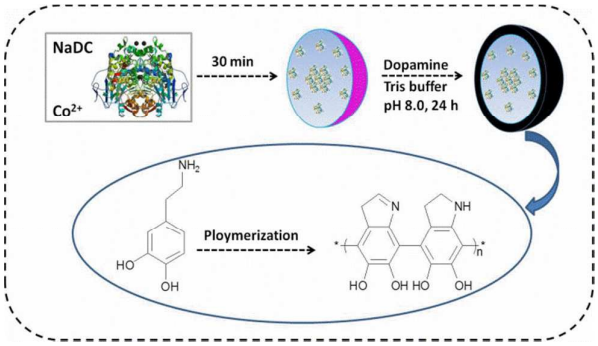


Fig. 1 Preparation of CRL-MSNC and PDA@CRL-MSNC.

mass spectrometry (ICP-MS) at 1.4 mg/g and 0.7 mg/g, respectively, with the content of cobalt in CRL-MSNC and PDA@CRL-MSNC at 62.4 mg/g and 30.8 mg/g, respectively. The amount of protein encapsulated in the CRL-MSNC and PDA@CRL-MSNC was 16 mg/g and 8 mg/g, respectively. Based on these analysis, we calculated that the molar ratio of Co: Na: DC: enzyme in the CRL-MSNC and PDA@CRL-MSNC was about $1.06{:}0.06{:}2.35{:}2.39\times10^{-4}$. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images (Fig. 2a) revealed a typical rod-shaped morphology for the metal ion-surfactant nanocomposites, with sizes of ~20 nm × 100 nm in the absence of enzyme. In the presence of enzyme, the as-prepared CRL-MSNC presented as nanospheres, with an average diameter of ~80 nm (Fig. 2b). After coating the CRL-MSNC with PDA, the PDA@CRL-MSNC presented an average diameter of ~100 nm (Fig. 2c), suggesting that the thickness of the PDA coating was ~20 nm. High-resolution TEM with energy-dispersive X-ray spectroscopy (EDS) mapping (Fig. 2d, inset) showed that protein (containing P element) was successfully embedded in the PDA@CRL-MSNC containing a cobalt ion. Based on linear distribution analysis of these molecules along the diameter of the particle, we observed that the protein molecules were mostly encapsulated inside the nanospheres, suggesting that during CRL-MSNC formation, the protein molecules might first aggregate with $Co^{2+}$ ions to form the core of the complexes.

Compared with CRL-MSNC, the Fourier transform infrared (FTIR) spectra of PDA@CRL-MSNC (Fig. S1) displayed an enhanced adsorption peak at 1600 $cm^{-1}$ that represented the C–C vibration of the benzene rings in PDA.[19] Thermal gravity analysis (TGA) also confirmed that after coating with PDA, the second decomposition stage of PDA@CRL-MSNC (starting from 130°C) was higher than that of MSNC and CRL-MSNC (starting from 100°C) (Fig. S2).

The hydrolytic activities of lipase in the CRL-MSNC and PDA@CRL-MSNC in aqueous solution were determined via the standard method using p-nitrophenyl butyrate (pNPB) as the substrate. As shown in Fig. 3a, CRL-MSNC retained ~100% of the activity of free lipase in solution, suggesting that the encapsulation of lipase in metal ion-surfactant complexes did not affect the catalytic configuration of lipase or impose substrate mass-transfer limitations. The activity of CRL-MSNC prepared by using other metal ions indicated that CRL-MSNC synthesized from $Co^{2+}$ had the highest activity (Fig. S3). This is probably due to the fact that $Co^{2+}$ has a strong coordination interaction with DC and therefore less $Co^{2+}$ can bind with enzyme molecules compared to other metal ions. The strong binding of metal ions with enzyme molecules usually causes the deactivation of enzyme. After applying the PDA coating, the activity of PDA@CRL-MSNC was slightly reduced at ~97% of the activity of free lipase in solution. This slight decrease in activity was likely due to the increased substrate mass-transfer limitation across the PDA shell. Normally, due to the loss of catalytic configuration and/or the significant mass-transfer limitation within immobilization carriers, the immobilized enzyme usually displays much lower activity relative to that of free enzyme in solution (usually <~70%).[20-22] Here, nanoscale encapsulation of an enzyme using relatively soft metal ion-surfactant complexes that neither

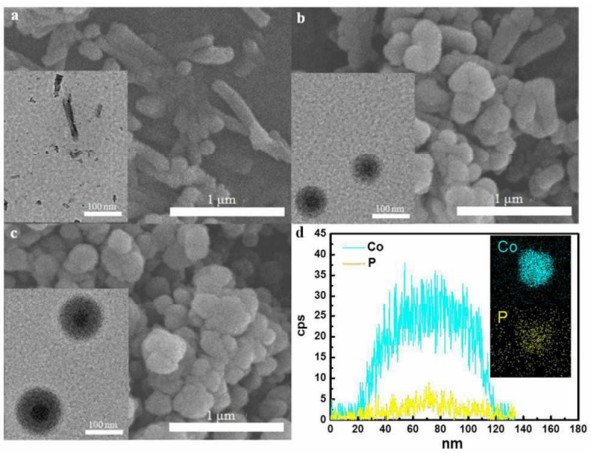


Fig. 2 (a) SEM and TEM images of MSNC. (b) SEM and TEM images of CRL-MSNC. (c) SEM and TEM images of PDA@CRL-MSNC. (d) EDS mapping of PDA@CRL-MSNC under TEM.

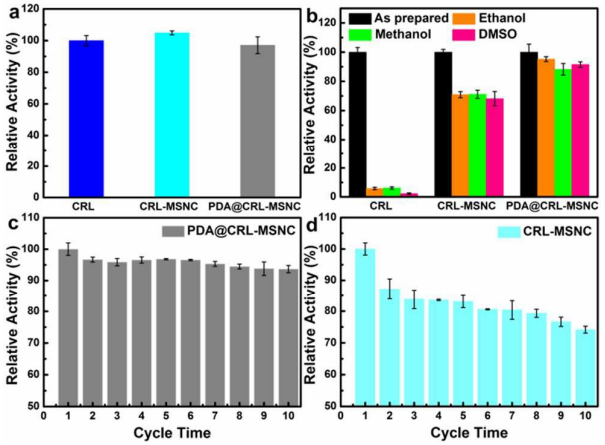


Fig. 3 (a) Relative activities CRL-MSNC and PDA@CRL-MSNC compared to free CRL. (b) Relative activities of CRL, CRL-MSNC, and PDA@CRL-MSNC after incubation in ethanol, methanol, and DMSO. Recycling use of (c) PDA@CRL-MSNC and (d) CRL-MSNC in aqueous media.

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affect the configuration of the protein nor impose obvious mass-transfer limitations, we observed nearly the same levels of enzyme activity as that of the free enzyme in solution. The high degree of retained enzyme activity confirmed our hypothesis that by using metal ion-surfactant complexes, the encapsulated enzyme can exhibit higher levels of activity as compared with other enzymes encapsulated within inorganic nanocrystals and metal-organic frameworks.[3,5] And PDA@CRL-MSNC has similar pH stability with native CRL (Fig. S4).

Free enzymes usually undergo denaturation in highly polar organic solvents, resulting in their exhibiting almost no activity following exposure to these solvents, such as ethanol, methanol, or dimethyl sulfoxide (DMSO).[23–25] The poor stability of enzymes in polar organic solvents limits their application in reaction mediums often necessary for simultaneously dissolving both hydrophobic and hydrophilic substrates. In this study, we observed that the CRL-MSNC and PDA@CRL-MSNC retained >68% and >88% of their original activities, respectively, following incubation in pure ethanol, methanol, and DMSO for 5 min at room temperature (Fig. 3b). By contrast, free lipase exhibited no activity under the same conditions. The greatly enhanced enzyme stability displayed by CRL-MSNC and PDA@CRL-MSNC in polar organic solvents was comparable to that of enzymes incorporated in metal-organic frameworks.[5,6] One possible explanation of the high degrees of enzyme stability in polar organic solvents is that the encapsulation of enzymes in hydrophilic nanoconfined spaces enhances protection of the protein structure against the denaturing effect of polar organic solvents, thereby allowing the retention of essential water molecules the protein surface.[26,27] Moreover, after PDA coating, the PDA@CRL-MSNC retained >93% of the original activity after 10 cycles of reuse in aqueous solution, suggesting a higher degree of reusability for PDA@CRL-MSNC as compared with CRL-MSNC (Fig. 3c and d).

The high degree of stability of CRL-MSNC and PDA@CRL-MSNC allowed us to investigate their application for the lipase-catalyzed synthesis of vitamin E succinate in organic solvents (Fig. 4a). This reaction requires the use of highly polar organic solvents to dissolve the substrates. Here, we first investigated the influence of polar organic solvents [including ethanol, acetonitrile, methanol, dimethylformamide (DMF), and DMSO] on the reaction catalyzed by free CRL (see the ESI† for experimental details). As shown in Fig. S5, the yield of vitamin E succinate was higher in DMSO as compared with that observed in other solvents, including ethanol, acetonitrile, methanol, and DMF (~12% conversion of vitamin E at a substrate molar ratio of 1:5 at 55°C for 4 h). Under the same conditions and using CRL-MSNC and PDA@CRL-MSNC as the catalysts (Fig. 4b), conversion of vitamin E reached ~55% after 4 h at 55°C, which was >4-fold higher than that observed for free CRL. Additionally, compared with commercially available lipase catalysts, such as Lipozyme *Thermomyces lanuginosus* lipase (TLIM) and Novozyme 435 (immobilized lipase), CRL-MSNC and PDA@CRL-MSNC exhibited enhanced levels of substrate conversion.

We then investigated the recyclability and long-term use of CRL-MSNC and PDA@CRL-MSNC for the aforementioned reaction. As shown in Fig. 4c, the relative activity of PDA@CRL-MSNC decreased by 55% after 10 rounds of use, suggesting adequate reusability for

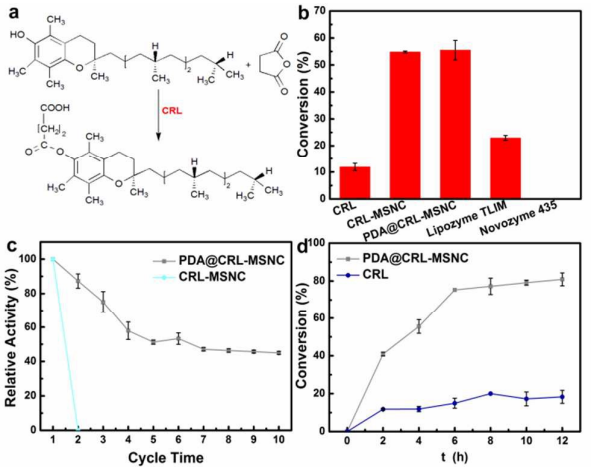


Fig. 4 (a) CRL-catalyzed synthesis of vitamin E succinate. (b) Synthesis of vitamin E succinate by different types of lipase. (c) Reusability of CRL-MSNC and PDA@ CRL-MSNC in DMSO. (d) Effect of reaction time on the synthesis of vitamin E succinate catalyzed by PDA@CRL-MSNC and free CRL.

this reaction system. By contrast, CRL-MSNC lost most of its activity after only one use, primarily due to the disassociation of CRL-MSNC in DMSO after >30 min (Fig. S6). The PDA coating on the CRL-MSNC created more covalent and noncovalent interactions between the PDA shell and the CRL-MSNC, which produced insoluble and stable black nanoparticles (Fig. S6) and strengthened the structural stability of the biocatalyst in DMSO. Comparison of time-course experiments measuring the synthesis of vitamin E succinate catalyzed by PDA@CRL-MSNC and free CRL (Fig. 4d) revealed that use of free CRL as the catalyst resulted in <20% conversion after 12 h due to the poor stability of CRL in DMSO. A previous study[18] also reported a slow reaction rate for the synthesis of vitamin E succinate using CRL as the catalyst (a yield of 46.95% after 18 h), supporting the poor stability of CRL in DMSO. Using PDA@CRL-MSNC as the catalyst increased the initial reaction rate 4-fold as compared with that observed using free CRL, with a conversion rate of ~80% after a 6-h reaction.

In conclusion, we described a co-precipitation method to encapsulate enzymes in metal ion-surfactant nanocomplexes. These complexes can be further cross-linked with PDA to form a highly effective nanobiocatalyst capable of exhibiting both high levels of activity and stability, especially in protein-denaturing polar organic solvents. This new type of lipase-encapsulated metal ion-surfactant nanocomposite exhibited much higher catalytic capability, stability, and reusability in the presence of polar organic solvents as compared with free lipase and commercially available immobilized lipases, thereby demonstrating its potential for applications requiring enzymatic catalysis under harsh conditions.

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