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Enhancement of the activity of enzyme immobilized on polydopamine-coated iron oxide nanoparticles by rational orientation of formate dehydrogenase

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ABSTRACT

Immobilization of enzymes onto nanoparticles and retention of their structure and activity, which may be related to the orientation of enzymes on nanoparticles, remain a challenge. Here, we developed a novel enzyme-orientation strategy to enhance the activity of formate dehydrogenase immobilized on polydopamine-coated iron oxide nanoparticles *via* site-directed mutation. Seven mutants were constructed based on homology modeling of formate dehydrogenase and immobilized on polydopamine-coated iron oxide nanoparticles to investigate the influence of these mutations on immobilization. The immobilized mutant C242A/C275V/C363V/K389C demonstrated the highest immobilization yield and retained 90% of its initial activity, which was about 3-fold higher than that of wild-type formate dehydrogenase. Moreover, co-immobilization of formate dehydrogenase and leucine dehydrogenase was performed for the synthesis of L-*tert*-leucine. The catalytic efficiency of the co-immobilized mutant C242A/C275V/C363V/K389C and leucine dehydrogenase increased by more than 4-fold compared to that of co-immobilized wild-type formate dehydrogenase and leucine dehydrogenase.

1. Introduction

Since most of the known oxidoreductases require expensive cofactors (NAD(H) or NADP(H)) for their catalytic activity, many methods have been developed for *in situ* cofactor regeneration (Liu and Wang, 2007; Schrittwieser et al., 2011; Wu et al., 2013). NAD-dependent formate dehydrogenase (EC 1.2.1.2) is one of the best candidates for the regeneration of NADH (Juan et al., 2006). To facilitate the reuse of the expensive cofactors and enzymes, some methods were developed to immobilize this regeneration system by covalent linking or physical entrapment (Mateo et al., 2010; Maurer et al., 2003; Rocha-Martín et al., 2012). However, in most of the immobilization methods, the binding sites between enzymes and carriers are random, and the supports are usually modified by chemical methods. Therefore, there is still a need for designing simple, orientable, and effective immobilization methods.

Recently, inspired by catechol-rich adhesive proteins secreted by marine mussels, catechol-derived compounds have been generating renewed interest because of their remarkable ability to strongly adhere to various inorganic and organic materials (Lee et al., 2007). Various catechol-modified magnetic nanocomposites were synthesized and applied for immobilization of biocatalysts, including lipase (Ren et al., 2011) as well as whole-cells of *Gluconobacter oxydans* (Ni et al., 2012). Specifically, the polydopamine-coated iron oxide nanoparticles (PD-IONPs) allowed the biocatalysts to be immobilized onto the surface with high loading-capacity efficiency and activity recovery under aqueous conditions.

In our previous work, formate dehydrogenase (FDH) from *Lodderomyces elongisporus* NRRL YB-4239 and leucine dehydrogenase (LDH) from *Bacillus subtilis* BEST7613 were co-immobilized onto PD-IONPs for synthesis of L-*tert*-leucine. The results indicated that LDH retained the desired activity. However, FDH had only a marginal activity after immobilization. Homology modeling of FDH revealed that there are 3 cysteine residues near the active site (Fig. 1A). Although the polydopamine can react with both thiol and amine groups (Lee et al., 2009), our research found that the thiol group reacts with polydopamine preferentially under neutral pH conditions (data not shown). Therefore, the relatively low activity recovery of co-immobilized FDH may be caused by the multiple covalent linking between polydopamine and the 3 cysteine residues. In this study, in order to improve the activity of the

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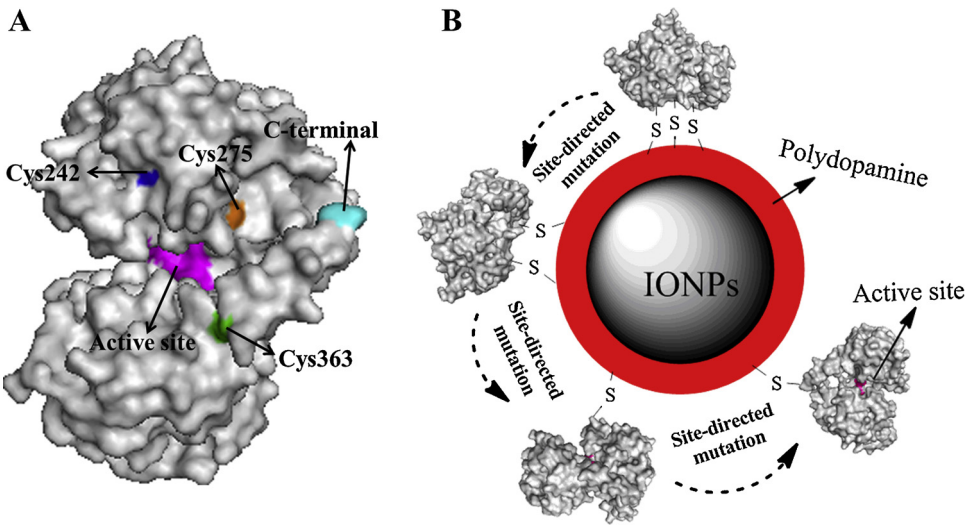

Fig. 1. (A) Homology modeling of formate dehydrogenase (FDH). (B) Schematic representation depicting the control of the orientation of formate dehydrogenase on PD-IONPs. The magenta region represents the active site, which was blocked in wild-type FDH when immobilized onto PD-IONPs. After rational control of the orientation of FDH by changing the location of specific cysteine residues through site-directed mutation, the active site was exposed gradually. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

immobilized enzyme and the immobilization yield, 7 mutants of FDH were constructed according to homology modeling, and the effects of these mutations on immobilization were investigated.

2. Materials and methods

2.1. Materials and reagents

The plasmid pET21a-LPFDH (containing FDH gene from *L. elongisporus* NRRL YB-4239, GenBank accession no. XM_001525495) and pET28a-BsLDH (containing LDH gene from *B. subtilis* BEST7613, GenBank accession no. BAM52893) were obtained from our laboratory. Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), dopamine hydrochloride, and NAD(H) were purchased from Sigma–Aldrich (Shanghai, China). All the other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and used without further purification.

2.2. Homology modeling and site-directed mutagenesis

The homology model of FDH was constructed using a template with high homology (CbFDHK47E, PDB ID 2FFS), following the protein modeling protocol of Discovery studio 2.5 (NeoTrident). The site-directed mutagenesis was performed using the KOD-plus-mutagenesis kit (Toyobo, Osaka, Japan), following the instructions of the manufacturer. The amino-acid residues replacing the cysteine residues were selected from a sequence comparison of different protein sequences of NAD-dependent FDHs (Fig. S1). The primers and templates of mutants were listed in Table S1.

2.3. Expression and purification

The recombinant *E. coli* BL21 (DE3) strains were cultured in LB medium containing 100 μg/mL ampicillin at 37 °C. When the OD600 reached 0.7, IPTG was added at a concentration of 0.1 mM and the cells were then incubated at 18 °C for 20 h. Further, the cells were harvested by centrifugation at 10,000 rpm for 10 min and resuspended in 20 mM PBS (pH 7.0). The resuspended cells were lysed by sonicating for 15 min at 4 °C. After centrifugation at 10,000 rpm for 30 min, the supernatant was then applied to an AKTA Prime system equipped with a 5-mL HisTrap™FF column (GE Healthcare, Waukesha, WI, USA). The results of expression and purification of proteins were checked by SDS-PAGE (Laemmli, 1970). As shown in Fig. S2, wtFDH and its mutants have similar expression and purity levels. Protein concentration was estimated using Bradford protein assay (Bradford, 1976).

2.4. Synthesis and characterization of PD-IONPs

PD-IONPs were prepared as previously described (Ren et al., 2011), with slight modifications. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were acquired on a HitachiHD2300 electron microscope (Hitachi, Japan) operated at 200 kV. TEM specimens were prepared by casting drops of dilute dispersion of nanoparticle aqueous solution on 200-mesh carbon-coated copper grids (Ted Pella).

2.5. Immobilization of FDH and its mutants

The immobilization was carried out by adding 1 mL (1 g/L) PD-IONPs solution to 3 mL PBS (pH 7, 20 mM) containing 1 mg pure enzyme. After subjecting the mixture to agitation at 200 rpm for 30 min at 4 °C, the precipitates were collected, washed 3 times, and stored at 4 °C prior to use. The loading of enzyme onto the PD-IONPs was calculated by using the difference between the protein concentration of the enzyme solution before and after immobilization. The immobilization yield is expressed as the percentage of bound protein relative to the protein offered to the carrier.

2.6. Activity assay

One unit (U) of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of NADH per min under the standard assay conditions. The standard assay mixture contained 50 mM ammonium formate, 0.1 mM NAD, 100 mM phosphate buffer (pH 7.5), and appropriate amount of enzyme/immobilized enzyme. After reaction at 37 °C for 15 min at 200 rpm, the increase in absorbance at 340 nm was monitored by using a SpectraMax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Specific activity is expressed as U/mg enzyme.