Journal of Biotechnology 241 (2017) 33–41

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

# Encapsulation of amine dehydrogenase in hybrid titania nanoparticles by polyethylenimine coating and templated biomineralization

Hong Ren[a], Yonghui Zhang[a], Jieru Su[a], Peng Lin[a], Bo Wang[a], Baishan Fang[a,b,*], Shizhen Wang[a,b,*]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China
[b] The Key Lab for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen, Fujian, 361005, PR China

ARTICLE INFO

*Article history:*
Received 18 October 2016
Received in revised form 4 November 2016
Accepted 7 November 2016
Available online 9 November 2016

*Keywords:*
Amine dehydrogenase
Polyethylenimine
Hybrid immobilization
Thermostability
Bioinspired mineralization
Multimeric enzyme

ABSTRACT

Asymmetric synthesis of chiral amines by amine dehydrogenases (AmDH), which catalyzed reductive amination of ketones with high enantioselectivity, is an ideal route for production of chiral amine. In this study, a facile approach is proposed to immobilize unstable amine dehydrogenase by two steps. Firstly, polyethylenimine (PEI), a cationic polymer, was applied for coating enzyme to inhibit the dissociation of multimeric enzymes. Strong interaction of PEI with AmDH were analyzed and indicated PEI provided the hydrophilic microenvironment for enzyme. The half-life was improved about 18 fold in 50 °C. Secondly, to further stabilize AmDH-PEI, PEI coated on AmDH surface was used as a biological template inducing the hydrolysis and condensation of titanium precursor to form titania. AmDH-PEI-Ti possessed more than 80% of the catalytic activity of free enzyme and the entrapment efficiency can be high up to 90%. A mechanistic illustration of the formation of AmDH-PEI-Ti nanoparticles were proposed. Titania provided a rigid cage pocket for the protection from structure unfolding. This generally applicable strategy offers a potential technique for multimeric enzyme immobilization with the advantages of low cost, easy operation, high reservation of activity and high stability.



## 1. Introduction

Chiral amines are key intermediates for pharmaceuticals and fine chemicals. The asymmetric synthesis of amines from prochiral ketones and free ammonia has been reported as one of the principal aspirational reactions challenging the pharmaceutical industry (Dunn et al., 2007). The application of lipases on the resolution of chiral amines has been restricted by its intrinsic efficiency of kinetic resolution which can only reach 50% (Mahmoudian, 2009). ω-Transaminases exhibit good enantioselectivity, while influenced by reaction kinetics balance and the substrate inhibition (Koszelewski et al., 2008). Amine dehydrogenases (AmDHs) are a new class of enzymes that have recently been obtained by protein engineering of wild-type amino acid dehydrogenases (Abrahamson et al., 2013) and chimeric amine dehydrogenase (Wu et al., 2011). In contrast to the lipases and transaminases which were widely applied in biosynthesis of chiral amine, AmDH dependent on NADH which catalyzed reductive amination of ketones with high enantioselectivity has the advantage such as easy co-enzyme regeneration. When paired a co-enzyme regeneration system with alcohol dehydrogenase (ADH), amine dehydrogenase implemented a redox self-sufficient reaction with high atom efficiency which generated water as the sole by-product (Mutti et al., 2015). Consequently asymmetric synthesis of chiral amines by amine dehydrogenases would be the ideal route to produce chiral amines. However, a pronounced loss of structure occurred in the circular dichroism spectroscopy of AmDH at temperatures above 30 °C, therefore the AmDH is unstable (Abrahamson et al., 2013). Several strategies are currently available for increasing operational stability such as addition of stabilizing additives, chemical modification, immobilization, and medium engineering (Iyer and Ananthanarayan, 2008).

Polyethylenimine (PEI) has been reported to prevent dissociation of enzyme multimers, by coating its surface with a polyionic polymer that may simultaneously interact with several enzyme subunits to prevent enzyme dissociation (Poltorak et al., 1998). PEI can interact with enzyme multimers within a certain distance and cover large protein surface, which achieved a complete crosslinking of subunits (Fuentes et al., 2004). Garcia-Galan et al. (Garcia-Galan et al., 2013) have researched that stabilization of glutamate dehy-

* Corresponding authors at: Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China
*E-mail addresses:* fbs@xmu.edu.cn (B. Fang), szwang@xmu.edu.cn (S. Wang).

http://dx.doi.org/10.1016/j.jbiotec.2016.11.006
0168-1656/

drogenase (GDH) which is a hexameric protein was increased and the activity was maintained in the presence of polyethyleneimine (PEI) because subunit dissociation was restricted. Researchers also have found that PEI-based cross-linking integrated into conventional cross-linked enzyme aggregates (CLEAs) from lipase applied in hydrolysis of fish oil (Yan et al., 2012). PEI coating is also applied for immobilization of glycerol dehydrogenase with the improvement of metal ions coordination and our research has been published (Bastakoti et al., 2014). PEI has been reported as a biological template for silica biomineralization either (Jin and Yuan, 2005).

Titania-based materials have received increasing interest for a wide range of applications, due to their excellent pH and thermal stability (Li et al., 2014), low toxicity (Wu et al., 2011), superior mechanical strength (Bastakoti et al., 2014), and biocompatible properties (Chen and Mao, 2007; Thompson and Yates, 2007). Yuan et al. (Jian-Jun and Ren-Hua, 2010) found that PEI is capable of templating and catalyzing the hydrolysis and subsequent polycondensation of titanium precursor to form titania. Moreover, the biomimetic synthesis of titania nanoparticles *in vitro* induced by protamine was investigated, and a relevant mechanism was tentatively proposed (Jiang et al., 2008). However, to date, there are no reports describing the biomimetic formation of titania induced by oxidoreductase coated with PEI for immobilization.

A simple and easy approach was proposed to prepare organic-inorganic hybrid enzyme nanoparticle. Positively charged polyethylenimine (PEI) was coated on the negative surface of amine dehydrogenase, and then the AmDH-PEI was applied as biomimetic template to induce the hydrolysis and polycondensation of titanium precursor to form nanoparticles (Jian-Jun and Ren-Hua, 2010). Morphological and functional studies of the enzyme immobilization by the PEIs induced biomimetic titanification were performed and the mechanism of the improvements in enzyme stability was studied.

## 2. Materials and methods

### 2.1. Chemicals

$NAD^+$, NADH, titanium (IV) bis (ammonium lactato) dihydroxide (Ti-BALDH, 50 wt% aqueous solution), polyethyleneimine (branched, MW: 25 KD) and isopropyl-beta-D-thiogalactopyranoside (IPTG) were purchased from Sigma Chemical Company (Tianjing, China). Acetophenone, 4-methyl-2-pentanone, 1,3-dimethylbutylamine, phenoxy-2-propanone, α-methylbenzylamine, and 3-methyl-2-butanone were also obtained from Sigma Chemical Company (Tianjing, China). The LB media were purchased from Sangon Biotech (Shanghai) Co. Ltd. All other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

### 2.2. Strains and culture conditions

AmDH gene was obtained from genetic modification of phenylalanine dehydrogenase from *Bacillus badius* (Abrahamson et al., 2013), which was sequenced and synthesized by Sangon BiotechCo. Ltd. (Shanghai, China). AmDH was expressed in a pET28a, BL21 (DE3) system with a C-terminal His-tag and cultured in LB media supplemented with 50 μg/mL kanamycin at 37 °C until OD600 reached 0.6. Isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM and incubated 8 h at 25 °C to induce protein expression.

### 2.3. Enzyme extraction and purification

The cells were centrifuged at 8000 rpm for 10 min, washed twice with Tris-HCl buffer (10 mM, pH 7.4, 4 °C) and resuspended. The cells were then pretreated by ultrasonication for 20 times for working 3 s and cooling for 3 s in an ice bath. To remove cell debris, samples were centrifuged at 10000 rpm for 15 min under 4 °C.

The crude extract was filtered through a membrane filter (0.22 μm) and loaded onto a 5 mL His-Trap HP affinity column. Ten column volumes of binding buffer (20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4) were applied to wash unbound impurities. The column was equilibrated with binding buffer and eluted with elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4) at a gradient concentration. The fractions with the desire activity were desalted and concentrated using a Macrosep Advance Centrifugal Device (cut-off 10 kDa, Pall, East Hills, NY, USA). Protein concentrations were determined with a modified Bradford protein assay kit (Sangon Biotech Co. Ltd) using bovine serum albumin as a standard, as described by Bradford (Bradford, 2015).

### 2.4. Enzyme assay

Amine dehydrogenase (AmDH) catalyzed asymmetric reduction of ketone to chiral amine with NADH as cofactor (Fig. 1). Activity of AmDH was measured using Tecan Infinite M200 Pro Spectra Microplate Reader. Measurements were taken at 340 nm and the molar extinction coefficient of NADH (6.22 $mM^{-1}$ $cm^{-1}$) (Li et al., 2014) was used. One unit of AmDH activity was defined as the amount of enzyme necessary to oxidize 1 μmol of NADH per minute under the following conditions. Specific activity for encapsulated enzyme was defined as full enzyme activity in titania particles divide by the concentration of protein amine dehydrogenase in titania particles.

For reductive amination, reactions were performed in 200 mM $NH_4Cl/NH_3H_2O$ buffer at pH 9.6, with 0.1 mM NADH and 20 mM of substrate ketone, unless otherwise specified. The catalytic activity of AmDH for reductive amination towards the non-natural substrates, phenoxy-2-propanone, α-Methylbenzylamine, 5-Methyl-2-hexanon, 2-hexanone, 3-methylcyclohexanone, 4-methyl-2-pentanone, acetophenone, was evaluated and phenoxy-2-propanone was chosen as the representative. For oxidative deamination reactions were performed in 100 mM Gly/NaOH buffer at pH 10.0, with 2 mM $NAD^+$ with 10 mM of the representative amines 1,3-dimethylbutylamine. All reactions were performed at 25 °C unless otherwise specified. For these two reciprocal reaction, reactions were started by the addition of 20 μL enzyme solution and the volume of the reaction mixture was 220 μL in all cases. Experiments on enzyme activities were determined in 4 replicates. Two kinds of blank controls for reductive amination reaction were set, including reaction mixture (reaction buffer and coenzyme) with ketone or not. Assay mixtures lacking substrates, or the mixtures lacking cell-free extracts, failed to demonstrate any measurable activity.

### 2.5. Preparation of AmDH-PEI-Ti hybrid microcapsules

AmDH coated on PEI were studied under different PEI concentration and pH condition. The concentration and pH of PEI were determined by measuring activity of AmDH-PEI. Aqueous solution of AmDH was prepared at 10 mM Tris-HCl buffer (pH 7.4). 2 mL of AmDH solution (protein concentration was about 1 mg/mL) was mixed with 2 mL PEI solution at 4 °C and mixture was shaken constantly for 30 min. The encapsulation was initiated by the addition of 4 mL AmDH-PEIsolution into 4 mL different concentrations Ti-BALDH solution. Resultant precipitate was collected by centrifu-