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Preparation of cobalt nanoparticles from polymorphic bacterial templates: A novel platform for biocatalysis

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ABSTRACT

Nanoparticles have gathered significant research attention as materials for enzyme immobilization due to their advantageous properties such as low diffusion rates, ease of manipulation, and large surface areas. Here, polymorphic cobalt nanoparticles of varied sizes and shapes were prepared using *Micrococcus lylae*, *Bacillus subtilis*, *Escherichia coli*, *Paracoccus* sp., and *Haloarcula vallismortis* as bacterial templates. Furthermore, nine lipases/carboxylesterases were successfully immobilized on these cobalt nanoparticles. Especially, immobilized forms of Est-Y29, LmH, and Sm23 were characterized in more detail for potential industrial applications. Immobilization of enzymes onto cobalt oxide nanoparticles prepared from polymorphic bacterial templates may have potential for efficient hydrolysis on an industrial-scale, with several advantages such as high retention of enzymatic activity, increased stability, and strong reusability.

1. Introduction

Enzymes are widely utilized in myriad industrial applications such as catalysis, medical diagnostics, water purification, bioelectronics, food processing, biomass conversion, drug delivery, and tissue engineering [1–3]. In comparison to inorganic or organometallic catalysts, the use of enzymes confers high level of specificity in chemical reactions. However, free enzymes pose numerous drawbacks such as lack of long-term stability, unwanted proteolysis, difficult separation, and poor recyclability. Most of these obstacles can be circumvented by immobilization of enzymes onto solid materials. To date, solid materials with a variety of morphological features, compositions, hydrophobicity, particle sizes, specific surface areas, functional surface groups, and rigidity have been utilized for enzyme immobilization [4,5]. Additionally, different immobilization methods based on physical adsorption, electrostatic forces, specific recognition, and covalent bonding have been employed [6,7]. The successful development of an immobilized enzyme depends primarily on the type of support material and the immobilization process, in addition to the enzyme properties [8]. In recent years, nanoparticles have been effectively developed for these purposes with excellent mechanical properties, morphological features, or physical-chemical characteristics [9,10].

As an extension of our study on enzyme engineering for biotechnological and industrial applications [11,12], we report herein the immobilization of microbial enzymes on polymorphic cobalt oxide nanoparticles prepared using bacterial templates. The fabrication of polymorphic nanostructures on the bacterial surfaces of *Micrococcus lylae*, *Bacillus subtilis*, *Escherichia coli*, *Paracoccus* sp. and *Haloarcula vallismortis* enabled the synthesis of a variety of cobalt oxide nanoparticles [13–15]. In this study, lipases/carboxylesterase were chosen for immobilization owing to their wide range of industrial applications including biodiesel formation, kinetic resolutions, and drug preparations [16–18]. Catalytic activity of the immobilized enzymes (*Ef*EstA, Est25, Est-Y29, P229, PA27, Sm23, Sm25, LI22, and LmH) was determined under different conditions. In addition, immobilized Est-Y29 was characterized in more detail for potential industrial applications. Furthermore, operational stability and recyclability of LmH, and Sm23 were also monitored. Our results indicate that in comparison to free enzymes, lipase/carboxylesterases immobilized on polymorphic cobalt oxide nanoparticles could be effectively used for biotechnological applications with enhanced catalytic performances.

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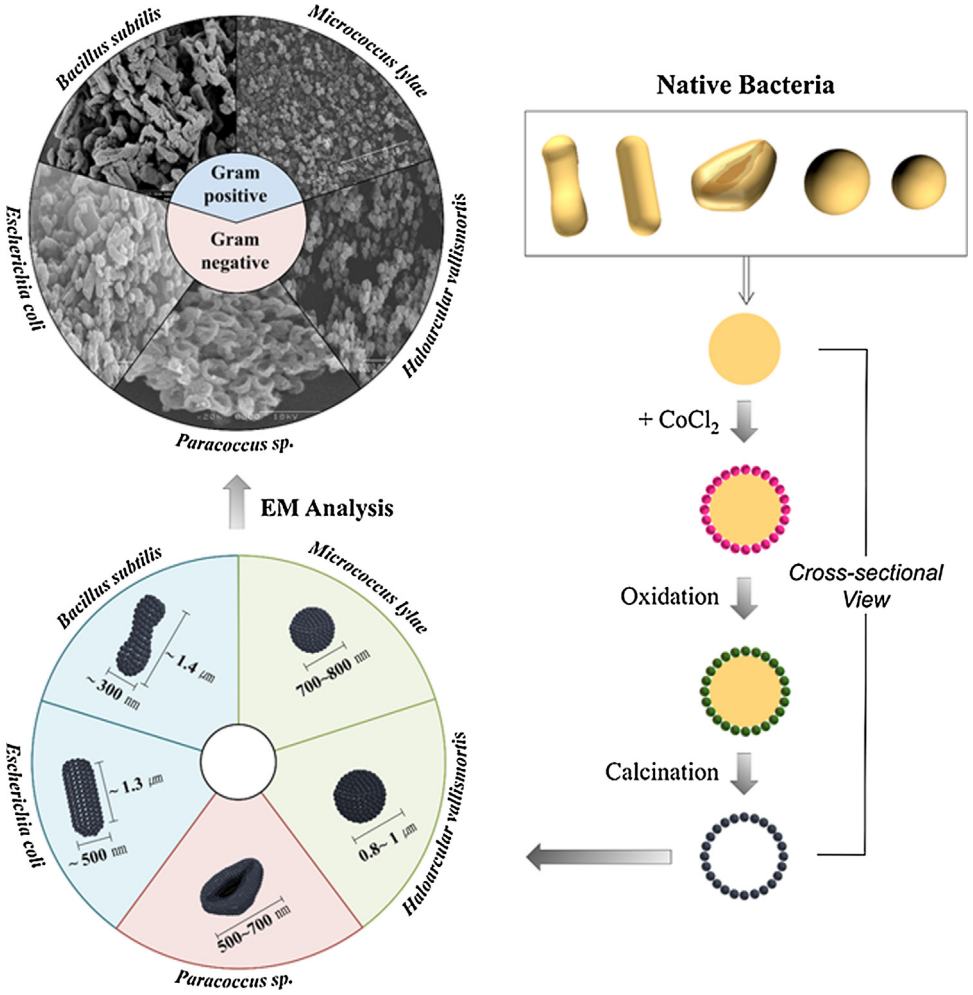

Scheme 1. Schematic representation of the synthesis of cobalt oxide nanoparticles using polymorphic bacterial templates. Electron microscopic images (upper left) and a model of the nanostructure (lower left) are shown for clarity. *M. lylae*, *B. subtilis*, *E. coli*, *H. vallismortis*, and *Paracoccus* sp. were used for the preparation of cobalt oxide nanoparticles. Note that the bacterial templates could be effectively removed by calcination while retaining the original shape and size.

2. Materials and methods

2.1. *Bacterial strains* and culture conditions

Micrococcus lylae, *Bacillus subtilis*, *Escherichia coli*, *Paracoccus* sp., and *Haloarcula vallismortis* cells were obtained from the Korean Collection for Type Culture (KCTC, Korea). *H. vallismortis* was cultured in 1 L of a complex medium consisting of yeast extract (5 g), Tris-sodium citrate (12 g), $MgCl_2$ (40 g), KCl (4 g), $CaCl_2$ (0.4 g), and NaCl (250 g) [19]. Other bacterial species were cultured in Luria-Bertani (LB) broth. After 18 h of incubation, and final optical density at 600 nm (OD_{600}) of ~1.6, cells were harvested by centrifugation at 6000 rpm for 10 min.

2.2. *Synthesis of cobalt oxide nanoparticles*

Cobalt chloride ($CoCl_2{\cdot}6H_2O$; 50 mM) solution was added to each bacteria sample (*M. lylae*, *B. subtilis*, *E. coli*, *P.* sp., and *H. vallismortis*). This solution was reduced by addition of 50 mM $NaBH_4$ under gentle shaking with incubation of 12 h. The resulting nanoparticles were harvested by centrifugation at 6000 rpm for 15 min, and subsequently washed three times with distilled water. The nanoparticles were dried at 60 °C for 1 h followed by calcination at 300 °C for 12 h. Then, these nanoparticles were washed with 70% ethanol (EtOH) followed by a wash with 20 mM Tris–HCl, pH 8.0. Finally, the nanoparticle containing solutions were centrifuged at 13,000 rpm for 10 min, and purified nanoparticles were stored at room temperature until further use [12,15].

2.3. *Characterization of enzyme-cobalt oxide nanoparticles*

Nine lipase/carboxylesterases (EfEstA, Est25, LI22, LmH, P229, PA27, Sm23, Est-Y29, and Sm25), which were genetically modified to contain His_6-tags, were expressed in *E. coli*, and purified by Ni-chelate affinity chromatography as previously described [20–25]. Immobilization process was performed at 4 °C by incubating cobalt oxide nanoparticles (2.0 mg) with above purified enzymes (4.0 μg) with gentle shaking (30 rpm) for 12 h. The resulting enzyme-coated nanoparticles were washed three times with a washing solution (20 mM Tris–HCl, pH 8.0) to remove any non-adsorbed enzymes. The efficiency of immobilization was determined by measuring the amount of residual enzyme found in the supernatant using the Bradford protein assay. Typically, less than 3% of total enzymes remained in the supernatant, indicating suitability of the cobalt oxide nanoparticles as immobilization matrix. Scanning electron microscopy images were obtained using a field-emission scanning electron microscope (FESEM, AURIGA, Carl Zeiss, Germany) using an acceleration voltage of 15 kV (NICEM, Seoul, Korea). Dynamic light scattering (DLS) was performed using Zetasizer NanoS system (Malvern Instruments Ltd., Malvern, UK).