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Fabricating polystyrene fiber-dehydrogenase assemble as a functional biocatalyst

Hongjie An, Bo Jin*, Sheng Dai

School of Chemical Engineering, The University of Adelaide, 5005 SA, Australia

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ABSTRACT

Immobilization of the enzymes on nano-structured materials is a promising approach to enhance enzyme stabilization, activation and reusability. This study aimed to develop polystyrene fiber-enzyme assembles to catalyze model formaldehyde to methanol dehydrogenation reaction, which is an essential step for bio-conversion of CO_2 to a renewable bioenergy. We fabricated and modified electrospun polystyrene fibers, which showed high capability to immobilize dehydrogenase for the fiber-enzyme assembles. Results from evaluation of biochemical activities of the fiber-enzyme assemble showed that nitriation with the nitric/sulfuric acid ratio (v/v, 10:1) and silanization treatment delivered desirable enzyme activity and long-term storage stability, showing great promising toward future large-scale applications.

1. Introduction

Immobilization of enzymes on nano-structured surfaces has been recognized as a technically promising and cost-effective approach to enhance enzyme stability, activity and reusability [1]. The nanostructured materials offer several intrinsic advantages, such as the larger surface areas that allow a higher loading of enzymes and the enhanced mass and energy transfer efficiency in a bioreactor system. Nano-biocatalysis, in which enzymes are incorporated into nanostructured materials, has emerged as a rapidly growing area [2–4]. Recent development in nanotechnology has provided a wealth of diverse nano-scaled scaffolds that could be used as the support for enzyme immobilization. Those nanostructures, including nano-porous media, nano-fibers [5], nano-tubes and nano-particles, have manifested great efficiency in manipulating the nano-scale environment of the enzymes and thus promise exciting advantages for improving enzyme performances [6]. Nanofibers can create a microenvironment which could enhance the mass transfer of substrate from the reaction medium to the enzyme active sites [2]. Among the nanostructured materials examined for biocatalytical applications, nano-structured polymer fibers (NPF) offer many outstanding characteristics, including high enzyme loading capability and highly homogenous dispersion in liquid phase [7]. In addition, the high porosity and interconnectivity endow nano-structured fibers with lower hindrance for mass transfer. The NPF surfaces can be modified to improve enzyme stability and activity. The specific surface characteristics, discrete nanostructures, low costs and ease of fabrication provide exciting opportunities to develop a feasible technology for enzyme-based bioprocesses in the presence of NPF support.

Polystyrene (PS) is usually functionalized to make its surface capable of covalent conjugation with proteins. A number of studies have been reported on the PS surface modification by introducing new functional groups, such as hydroxyl, amino, carbonyl, and carboxyl to change its polarity and wettability, so that proteins can be easily grafted to the surface. In practical, those hydrophilic groups are introduced by the surface treatment of ion irradiation [8], plasma [9], electron beam or UV [10,11]. Page et al. modified the surface of PS beads by the nitrating process using sulfuric and nitric acids, followed by the reduction of nitro groups [12]. They confirmed the resulting amine groups were able to immobilize antibodies. Moreover, enzymes have also been reported to better retain their bioactivity as being immobilized via extended spacer arms using the one-step aqueous silanization chemistry to introduce amino groups to microtiter wells for cell growth [13].

Electrospun fiber has attracted an increasing research attention due to its various applications as biocatalyst scaffolds [5]. Electrospun polystyrene fibers (EPSNF) are desirable for enzyme immobilization due to their non-toxicity, low cost and good mechanical properties. To date, the functional groups of EPSNF used for enzyme immobilization are typically introduced by electrospinning the blender of PS and other polymers with functional

* Corresponding author. Tel.: +61 8 83137056; fax: +61 8 83036222.
E-mail address: Bo.Jin@adelaide.edu.au (B. Jin).

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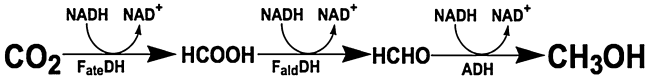

Scheme 1. Consecutive bioconversion of CO_2 to methanol catalyzed by three dehydrogenases.

groups such as —COOH [14]. However, the dispersion capability of such fibers in aqueous solution is limited, which significantly influence further enzyme loading and enzyme activity. There is almost no information available on the direct modification of pure EPSNFs as the biocatalytical scaffolds.

Obert and Dave reported an enzymatically coupled sequential conversion of CO_2 to methanol in a series of reactions catalyzed by three different dehydrogenases [15]. The process, as shown in Scheme 1, involves an initial reduction of CO_2 to formate catalyzed by formate dehydrogenase ($F_{ate}DH$), followed by reduction of formate to formaldehyde by formaldehyde dehydrogenase ($F_{ald}DH$), and finally formaldehyde is reduced to methanol by alcohol dehydrogenase (ADH). However, there are a few crucial technical barriers, including separation and recovery, poor stability/activation and product/intermediate contamination of these enzymes, making this multi-dehydrogenation process technically unreliable and economically unfeasible [16]. The high costs related to these dehydrogenases and cofactor (~$1500/mole of NADH) restricts their applications in an industrial process [17].

In this study, we investigated the EPSNF nitration in the presence of sulfuric and nitric acids. The influence of the acid mixing ratio on the EPSNF surface properties, enzyme loading efficiency, enzyme activity and stability was systematically examined. The modifications were characterized and the system was evaluated by determining the activity of alcohol dehydrogenate (ADH) in the bioconversion of HCHO to CH_3OH, which is one of three dehydrogenation reactions of bioconversion CO_2 into methanol (Scheme 1).

2. Materials and methods

2.1. Electrospinning

PS stock solutions (20 and 30%, w/w) were prepared by dissolving PS (MW ~350,000, Aldrich) in *N*,*N*-dimethylformamide (DMF, 98%, Sigma–Aldrich). The solutions were shaked at 50 rpm on an orbital shake incubator at room temperature overnight to ensure PS was fully dissolved. The electrospinning of PS fibers was conducted at room temperature. 0.5 mL of PS stock solution was loaded into a 1 mL syringe equipped with a 22-gauge needle. The syringe was horizontally fixed on a syringe pump (NE-300, New Era Pump Systems), and the solutions were electrospun using high voltage supply (Glassman, PS/EL30P01.5-22). Electrospinning process was conducted with a flow rate of 1 mL/h associated with a variety of operating voltages of 20, and 30 kV. A grounded aluminum foil collector was positioned 15 cm from the tip of the needle. After electrospinning, the fibers were dried at 60 °C in vacuum for 24 h before scanning electron microscope (SEM) examination.

2.2. Surface modification and silanization of electrospun PS fibers

The surface modification procedure of PS fibers was illustrated in the flowchart of Fig. 1. Electrospun PS fibers (200 mg) were nitrated with various mixtures of concentrated (63%) nitric acid and (98%) sulfuric acids (v/v, 1:1, 2:1, 3:1, 4:1, 5:1, and 10:1) for 2 h at room temperature under mild shaking (50 rpm), followed by the silanization process as described by Raman Suri and Kaur [18]. The nitro fibers were washed three to five times using Millipore deionized water till the pH of 7, and then treated with 2% (v/v) 3-aminoproplytrimethoxysilane (APTMS, Aldrich) aqueous solution at room temperature for 2 h under mild shaking. The resulting PS fibers were washed with deionized water and dried at 60 °C in vacuum for 2 h.

2.3. Enzyme immobilization and activity assay

After APTMS coating, PS fibers (60 mg) were equilibrated in phosphate buffer silane (PBS, 50 mM, pH 7.0) for 2 h, and then mixed with alcohol dehydrogenase (ADH (A7011), Sigma) solution (0.10 mg/mL) containing *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, Sigma) and *N*-hydroxysuccinimide (NHS, Sigma) (10 g/L in PBS, 50 mM, pH 7.0, molar ratio 1:1) at room temperature gently shaking for 12 h. The resulting ADH-immobilized fibers were washed with PBS until no protein was detected in the supernatant. The enzyme loading efficiency was determined by Bradford assay [19], where bovine serum albumin (Sigma) was used to prepare calibration curve. Enzymatic activity was evaluated by recording the change in absorption at 340 nm using UV–visible spectrophotometer (LIUV-201 Lambda Scientific). The assay system of ADH contained formaldehyde (5–50 μM), β-nicotinamide adenine dinucleotide phosphate (50–300 μM, Sigma), 0.1 M PBS (pH 7.0). The mixture (3 mL) was put into appropriate cuvettes, immediately mixed by inversion and recorded the absorbance A_{340} every 30 s, continuously for 5 min. For the fibrous enzymes, the reaction mixture containing fibers were put in a 50 mL centrifuge tube. A_{340} was measured every 30 s by pipetting 3 mL reaction solutions into a cuvettes, and then were returning into centrifuge tube after measurement. Thus the reduction of formaldehyde to methanol can be calculated by determining the amount of NADH consumption.

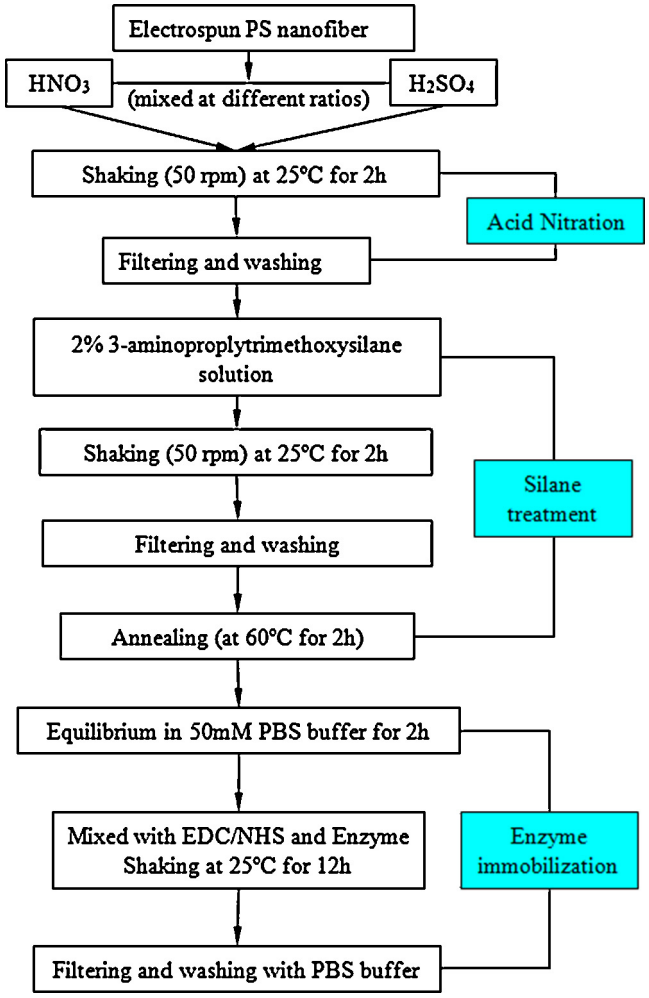

Fig. 1. Flow chart of the preparation of nano-structured polymer enzyme assembles for this study, which include PS fiber acid nitration, silanization, and enzyme immobilization process.

2.4. Characterization of PS fibers

Specimens of the electrospun PS fibers (with and without surface treatment) were analyzed by SEM (Philips XL 30 FEGSEM) associated with an energy-dispersive X-ray spectroscopy (EDXS), Fourier transform infrared spectroscopy (FTIR, Nicolet 6700) and Raman spectroscopy (PRO-785, PeakSeeker). Fluorescence DyLight™ 649 labeled donkey anti-rabbit IgG antibody (excitation 655 nm, and emission 670 nm) was used as a model protein to verify the immobilization capability of surface activated PS fibers using an Olympus BX51 fluorescence microscope.

3. Results and discussion

3.1. Electrospinning optimization

It has been reported that the morphologies and diameters of EPSNF can be tailored by varying polymer molecular weight (MW),