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Preparation and characterization of novel green magnetic nanocatalyst for cellulosic biomass degradation under mild conditions

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ABSTRACT

This paper describes the preparation and characterization of a green magnetic nanocatalyst (**GMN**) that is functionalized with carboxyl and imidazole groups, which hydrolyze β-1,4-glycosidic bonds with a high catalytic efficiency. The **GMN** mimics the structure of the active sites of polysaccharide-degrading enzymes under mild conditions (pH 5). The maximal glucose yield of 0.53 mg/mL and xylose yield of 0.17 mg/mL were obtained from the enzyme cocktail of **GMN** and cellulase. The **GMN** also retained a significant amount of activity after five consecutive reaction cycles.

Introduction

Lignocellulosic biofuels and materials have drawn increasing attention in recent years as alternative sources of energy to supplement conventional fossil fuels [1–3]. The bioconversion of lignocellulosic components into fermentable sugars is required to initiate the production of industrially important products from lignocellulosic biomass. This bioconversion process is much more complex than the fermentation of simple C6 sugars and is far from being cost-effective when compared with the production of bioethanol from starch or sugar crops [4–8].

The current approach for converting lignocellulosic biomass to ethanol depends largely on enzymes that degrade polysaccharides into fermentable sugars. This enzymatic hydrolysis requires mild reaction conditions, high hydrolysis activity, and high selectivity to produce reducing sugars [9–11]. Naturally occurring biocatalysts are environmentally friendly and boast high specificity with low consumption of energy and reagents [12–14]. Despite such advantages, the use of biocatalysts in industrial processes remains limited by several factors: most biocatalysts are relatively unstable at high temperatures; the costs of isolating and purifying biocatalysts remain high; and the recovery of biocatalysts from reaction mixtures is quite difficult. As a consequence, the use of native biocatalysts for broader industrial applications is often severely limited [15–20]. One approach to resolving these difficulties is to synthesize reusable biomimetic catalysts that are stable heterogeneous biocatalysts and are recoverable. Improvement of their recovery and operational stability would result in reduced costs and higher overall bioprocess efficiency.

Biomimetic catalysis refers generally to a form of chemical catalysis that mimics certain key features of native biocatalysts [21]. Biomimetic catalysts may be used repeatedly to yield considerable cost savings because they are separated easily from reaction mixtures, and are stable under harsh reaction conditions that would not be suitable for soluble enzyme preparations. Thus, developing an understanding of the structures and catalytic mechanisms of natural catalyst systems is important [22,23].

We reported previously that, compared with natural enzymes, a biomimetic magnetic nanoenzyme that was functionalized with a carboxylic acid group exhibited improved catalytic efficacy and repeatability in the hydrolysis of cellulose to reducing sugars at a pH of 3.0 [24]. The nanoenzyme catalyzed the hydrolysis through a general acid–base mechanism mediated by two carboxylic acid moieties [23,25]; however, its activity under low-pH conditions could be problematic for downstream operations. Enzymatic hydrolysis is carried out to degrade cellulose to reducing sugars,

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and ideally, it would occur under mild conditions such as pH 4.5–5.0 and temperature 45–50 °C [26]. A common feature of biocatalytic reaction mechanisms is cooperative catalysis by an acid–base pair on the side chains of two adjacent amino acids associated with the proper folding of a protein polymer. For example, aspartate–histidine pairs are critical constituents of several key enzymatic reactions [27,28]. Aspartate and histidine have a carboxylate and an imidazole group, respectively, on their side chains.

Factors including the lack of availability of hydrolytic enzymes in sufficient quantities, low enzyme stability, and substrate selectivity limit the potential use of natural hydrolases for a broader range of applications [20]. As a consequence, novel catalysts that can cleave β-1,4-glycosidic bonds under mild conditions are urgently needed. In this paper, we describe a novel green magnetic nanocatalyst (**GMN**) designated **GMN** that can be used to cleave cellulose to sugars under mild conditions of pH 5.0 and 37 °C. The biochemical properties, catalytic activity, and reusability of **GMN** are compared with those of natural catalysts.

Material and methods

Materials

Experimental materials including 4-aminobenzoic acid, 1-(3-aminopropyl)imidazole, 3-(triethoxysilyl)propyl isocyanate, *p*-nitrophenyl β-D-glucopyranoside were purchased from Sigma–Aldrich (USA). Cellohexaose was purchased from Seikagaku Corporation (Japan). All other materials were of analytical grade and available commercially, including ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ammonium hydroxide (NH_4OH, 25% [w/w]), tetraethyl orthosilicate (TEOS), tetrahydrofuran (THF), toluene, and chloroform ($CHCl_3$). Bio-Rad reagent for use in a protein assay was obtained from Bio-Rad Laboratories (Hercules, CA). Water used throughout this study was deionized and filtered using a U.S. Filter purification system.

Instruments

A 300 MHz Varian Unity spectrometer using tetramethylsilane (TMS) as an internal standard was used to record ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra. Infrared (IR) spectra were obtained in the 400–4000 cm^{-1} wavenumber range using a PerkinElmer FT-IR/NIR 400 instrument. Field-emission transmission electron micrographs (FE-TEM) were generated using JEOL JEM-1400 microscopes and a JEOL 100-CX electron microscope (Jeol, Ltd., Japan). High-resolution X-Ray diffractograms (HR-XRDs) were generated using an X'Pert PRO multipurpose X-ray diffractometer (X'Pert-Pro MPD, Philips). Thermal gravimetric analysis was conducted using a TGA-2950 thermogravimetric analyzer (Thermal Advantage, Inc.) at a heating rate of 10 °C/min using a Pt pan in air; the temperature was scanned from 25 to 600 °C. High-performance liquid chromatography (HPLC) was performed using a refractive index (RI) detector (Waters 2414, USA) equipped with a Rezex RPM column (4.6 mm × 300 mm; Phenomenex, USA). HPLC-grade water was supplied at a flow rate of 0.6 mL/min as a mobile phase at a controlled temperature of 80 °C.

*Synthesis of carboxylic acid derivative **1***

4-Aminobenzoic acid (0.5 g, 3.64 mmol) was dissolved in 20 mL of chloroform ($CHCl_3$) and 5 mL of tetrahydrofuran (THF) followed by drop-wise addition of 4.38 mmol (1.08 g) of 3-(triethoxysilyl)-propyl isocyanate to the solution with stirring. The mixture was heated at 80 °C in a round-bottomed flask for approximately 10 h. Cold hexane was added to yield a white powder precipitate. The powder was isolated via filtration, purified in hexane, and dried under reduced pressure. ^1H NMR (DMSO-d_6): δ 8.80 (s, 1H, –NH), 7.80 (d, 2H, ArH, J = 8.7 Hz), 7.48 (d, 2H, ArH, J = 8.7 Hz), 6.32 (t, 1H, –NH, J = 5.7 Hz), 3.75 (q, 6H, $-OCH_2CH_3$, J = 6.9 Hz), 3.07 (q, 2H, $-NHCH_2CH_2-$, J = 6.6 Hz), 1.49 (m, 2H, $-CH_2CH_2CH_2-$), 1.15 (t, 9H, $-OCH_2CH_3$, J = 6.9 Hz), 0.57 (m, 2H, $-CH_2CH_2Si-$); ^{13}C NMR (DMSO-d_6): δ 167.18 (–C═O), 154.82(–C═O), 144.92, 130.52, 122.78, 116.61, 57.78, 41.80, 23.27, 18.27, 7.29.

*Synthesis of imidazole derivative **2***

4-Aminobenzoic acid (0.5 g, 3.99 mmol) was dissolved in 20 mL of chloroform ($CHCl_3$) and 5 mL of methanol (MeOH), followed by the drop-wise addition of 1.19 g (4.38 mmol) of 3-(triethoxysilyl)-propyl isocyanate to the solution with stirring. The mixture was stirred for 24 h at 80 °C and then poured into 200 mL of distilled water. The aqueous phase was extracted with dichloromethane (CH_2Cl_2, 2× 200 mL) and the combined organic phases were dried over sodium sulfate (Na_2SO_4) and evaporated *in vacuo* to yield 0.52 g (76%) of **2**. ^1H NMR (DMSO-d_6, 300 MHz): δ 7.61 (s, 1H, ArH), 7.16 (s, 1H, ArH), 6.87 (s, 1H, NH), 5.86 (m, 2H, –NH), 3.93 (t, 2H, $-CH_2CH_2-$, J = 6.9 Hz), 3.74 (q, 6H, $-OCH_2CH_3$, J = 6.9 Hz) 2.93 (q, 4H, $-CH_2CH_2NH-$, J = 6.6 Hz), 1.78 (quin, 2H, $-CH_2CH_2CH_2-$, J = 6.9 Hz), 1.41 (m, 2H, $-CH_2CH_2CH_2Si-$), 1.12 (t, 9H, $-OCH_2CH_3$, J = 6.9 Hz), 0.51 (m, 2H, $-CH_2CH_2Si-$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 158.16 (–C═O), 137.27, 128.34, 119.35 (ArC), 57.72, 43.66, 42.09, 36.46, 31.76, 23.58, 18.23, 7.27.

*Preparation of the magnetic manoparticles (**MN**)*

The magnetic nanoparticles (**MN**) were prepared through the precipitation of $FeCl_3{\cdot}6H_2O$ (1.0 M) and $FeCl_2{\cdot}4H_2O$ (2.0 M), as indicated by Cho et al. [29].

*Preparation of the green magnetic nanocatalyst (**GMN**)*

MN (1.0 g) was resuspended in 50 mL of toluene under sonication for 30 min. The preparation method for **MN** is provided in the supporting information. Carboxylic derivative **1** (0.5 g) and imidazole derivative **2** (0.5 g) were added, and the reaction mixture was refluxed for 24 h. After cooling to room temperature, the particles were filtered and washed with toluene and then dried at 60 °C for 12 h in a vacuum oven.

*Effects of pH on **MN** and **GMN***

The optimum catalytic pH values for **MN** and **GMN** were determined using a citrate–phosphate buffer over a range of pH values from 2.0 to 7.0 in assays carried out for 15 min at 80 °C. The activities were determined by measuring the release of *p*-nitrophenyl from *p*-nitrophenyl β-D-glucopyranoside (*p*NPG). Hydrolysis reactions were performed using 300 μL of 10 mM *p*NPG and 20 mg of **MN** or **GMN** in a total volume of 1 mL. The reaction was stopped by adding 1 M sodium carbonate (Na_2CO_3), and the absorbance was measured at 405 nm.

Cellohexaose activity

Activity was ascertained by measuring the amount of reducing sugars released from a 1% cellohexaose solution in 100 mM sodium acetate buffer (pH 5.0) for 24 h at 50 °C. Hydrolysis reactions were performed using 30 μL of 10 mM cellohexaose solution and 20 mg of **GMN** in a total volume of 500 μL. The amount of reducing sugars was measured according to the dinitrosalicylic acid (DNS) method [30] and the monosaccharide composition in the supernatant was determined by HPLC.