

Short communication

Molecularly imprinted soluble nanogels as a peroxidase-like catalyst in the oxidation reaction of homovanillic acid under aqueous conditions

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Abstract

A novel type of soluble peroxidase-like imprinted nanogels incorporating hemin, acrylamide and 4-vinylpyridine as functional monomers was prepared by solution polymerization method. Such highly crosslinked nanogels could be conveniently separated from the reaction mixture through precipitation, ultracentrifugation or ultrafiltration, whereby they could be analyzed by gel permeation chromatography (GPC), transmission electron microscopy (TEM), dynamic light scattering (DLS) and environmental scanning electron microscope (ESEM). More importantly, the molecularly imprinted polymers (MIP) showed greater catalytic activity in DMSO–Tris–HCl buffer than in pure Tris–HCl buffer mainly because it could behave as a homogeneous catalyst under appropriate DMSO–Tris–HCl system. The results suggested that the unavoidably restrained transport properties inside conventional insoluble imprinted polymers might be overcome. Additionally, the MIP showed higher adsorption towards substrate under aqueous conditions compared with its corresponding blank polymer, which was due in part to its higher catalytic activity. © 2007 Elsevier B.V. All rights reserved.

Keywords: Molecularly imprinted polymers; Enzyme mimic; Nanogel; Peroxidase

1. Introduction

Molecular imprinting has been recently regarded as one of the most potentially promising methods to create a three-dimensional network with a memorized cavity specific to the shape and functional group positions of the template molecules [1–7]. However, two intrinsic difficulties presented a challenge to the advancement of molecular imprinting technology in mimicking enzyme models in water: one is that hydrogen-bonding interactions between polar functional groups are weakened by hydration when compared with the interactions in non-polar solvents; the other is the insolubility of these enzyme-like polymers and the heterogeneity of the active sites under aqueous conditions where biocatalytic reactions proceed.

Nanogels with diameters in the range of several to hundreds of nanometers are of wide interest in the fields of colloids and

polymers [8–10]. One of the main advantages of these nanogels is that they can form homogeneous colloidal solutions when dissolved in an appropriate solvent system. Graham and Cameron developed an elegant method to synthesis highly crosslinked nanogels in dilute solution [11]. Wulff's group obtained soluble highly crosslinked imprinted microgels by this method [12]. Recently, soluble catalytic imprinted microgels by Resmini and co-workers [13,14] and soluble imprinted nanogels with single cavity per particle for carbonate hydrolysis by Wulff et al. have been reported [15]. Homogeneous phase catalysis successfully proceeded in these models, making it possible to resolve the hamper of mass transfer in contrast to the existing of traditional insoluble imprinted polymers. However, these enzyme-like polymers did not show significant catalysis activities under aqueous media due to negative contribution of water molecules to molecular recognition.

In the previous work by our group, a peroxidase-like insoluble molecularly imprinted polymer has been reported which can efficiently catalyze the oxidation of homovanillic acid (HVA) with considerable substrate specificity in the presence of hydrogen peroxide [16,17]. By taking advantage of

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the unique structural features of hemin and multiple-site interactions provided by several functional monomers, the intrinsic difficulty for molecularly imprinted polymers (MIPs) in recognizing template molecules in polar solutions is likely to be resolved. In this paper, we will focus on preparing soluble molecularly imprinted nanogels containing hemin to improve the potential for higher catalytic activity under aqueous media.

2. Experimental

2.1. Chemicals

Ethylene glycol dimethacrylate (EDMA) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Toshima, Kita-ku, Tokyo). Acrylamide (AAm) was from Sangon Co. Ltd. and 4-vinylpyridine (4-Vpy) from E. Merck (Darmstadt, Germany). Azo-bisisobutyronitrile (AIBN) was obtained from Nankai Chemical Plant (Tianjing, China). Hemin, (*p*-hydroxyphenyl) acetic acid (*p*-HPA), and (*p*-hydroxyphenyl) propionic acid (*p*-HPPA) were from Sigma Chemical Co. (St. Louis, MO, USA), and homovanillic acid (4-hydroxy-3-methoxyphenyl acetic acid, HVA) from Kanto Chemical Co. Inc. (Chuo-Ku, Tokyo). Monomers and crosslinker were distilled immediately prior to use, and initiator AIBN was recrystallized from ethanol. All solvents and other chemicals were of analytical grade.

2.2. Preparation of molecularly imprinted nanogels

Polymer nanogels were prepared by radical solution polymerization [9,10]. Monomer concentration is about 1% (w/w) to prevent coagulation. In a typical experiment, after the dissolution of hemin (13 mg, 0.02 mmol), AAm (5.6 mg, 0.08 mmol), 4-Vpy (8 μ l, 0.08 mmol), HVA (3.5 mg, 0.02 mmol), EDMA (180 μ l, 0.95 mmol) and 10 mg of AIBN in 20 ml of DMSO, the mixture was bubbled with nitrogen for 10 min and heated to 70 °C to start the polymerization. The reaction proceeded under stirring (400 rpm) for 48 h at 70 °C. After cooling, the polymer was precipitated by the addition of diethylether, centrifugated at 12,000 rpm for 30 min and resuspended in a solution of 10% acetic acid in methanol (v/v) to extract the template [15]. The solution was gently stirred overnight. The particles were centrifugated again and thoroughly dialyzed (molecular weight cut off 3500; Visking membrane, Carl Roth GmbH, Germany) for 3 days against distilled water. Finally, the particles were freeze-dried in vacuum at -50 °C in an ALPHA1-4 freezer (MCG, Germany) for at least 48 h. The non-imprinted control polymer (NIP) was generated in the same way without the addition of HVA template.

2.3. GPC analysis

The gel permeation chromatography apparatus consisted of a Waters 510 pump, three columns (HT2, HT3, and HT4), and a Waters 2410 refractive index detector. Samples were taken from the solution by dissolving 20 mg of nanogel in 2 ml of THF. Polystyrene standards were used for the calibration, with THF as the mobile phase.

2.4. TEM

Transmission electron microscopy (TEM) experiments were carried out on H-9000NAR (Hitachi, Japan) instrument with an acceleration voltage of 100 kV. TEM sample was prepared by dipping a carbon coated copper grid into the freshly prepared nanogels solution in THF, which was subsequently air dried at room temperature before loading into the microscope.

2.5. Dynamic light scattering (DLS) studies

The hydrodynamic particle sizes were measured with quasielastic light scattering on a Brookhaven Instruments Corp. [18,19]. BI-200SM goniometer was equipped with a BI-9000AT digital correlator using a solid-state laser (125 mW, $\lambda = 532$ nm) at a fixed scattering angle of 90°. Light scattering measurements were made on dilute particle dispersions with concentrations of about 0.1 mg ml⁻¹ at 25 °C. The software (BIC particle sizing) derived particle sizes and size distributions from the correlator function.

2.6. ESEM

The particles morphology was observed on an environmental scanning electron microscope (ESEM) (FEI Quanta 200FEG). The particles containing water without drying were mounted on metal stubs and at low vacuum degree ($\sim 10^{-3}$ atm) and relatively low temperature (near 4 °C).

2.7. Determination of the hemin content present in the polymers

The hemin present in the polymers was measured by quantitative spectrophotometric (398 nm) analysis based on subtraction of the extractable hemin from the hemin added for the reaction [16,17].

2.8. Catalytic reaction with MIP as catalyst

HVA can be oxidized to a dimer by hydrogen peroxide with HRP as catalyst, and this dimer shows strong fluorescent emission under alkaline conditions (Ex/Em = 315/425 nm) [20,21]. In this work, the catalytic reactions were carried out in Tris-HCl buffer (50 mM, pH 8.5), stirred with the suspended solution of the polymers at 25 °C. The final concentrations were fixed at 5.0×10^{-2} g l⁻¹ (hemin concentration, 4.04×10^{-6} M) for the polymer and 5.26×10^{-3} M for H₂O₂, with varying HVA concentrations from 1.34×10^{-3} M to 1.10×10^{-4} M. The catalytic reactions were carried out in DMSO-Tris-HCl buffer (5/95, v/v, 50 mM, pH 8.5) at conditions as those in pure Tris-HCl buffer except that the final concentration of the polymer decreased to 5.0×10^{-3} g l⁻¹ (hemin concentration, 4.04×10^{-7} M) because the fluorescence intensity was too high to be measured. The dynamic curve of the dimerization reaction of HVA catalyzed by the MIP was recorded with a luminescence spectrometer (LS-50B, Perkin-Elmer, USA) and the pseudo-first-order rate constants were obtained from linear plotting of

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