Journal of Colloid and Interface Science 349 (2010) 98–105

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Laccase-modified silica nanoparticles efficiently catalyze the transformation of phenolic compounds

Patrick Galliker [b], Gregor Hommes [b], Dietmar Schlosser [c], Philippe F.-X. Corvini [b], Patrick Shahgaldian [a,*]

[a] Institute for Chemistry and Bioanalytics and School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Gründenstrasse 40, Muttenz CH-4132, Switzerland
[b] Institute for Ecopreneurship and, School of Life Sciences, University of Applied Sciences Northwestern Switzerland, Gründenstrasse 40, Muttenz CH-4132, Switzerland
[c] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research –UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

ARTICLE INFO

Article history:
Received 24 February 2010
Accepted 8 May 2010
Available online 16 May 2010

Keywords:
Laccase
Silica
Nanoparticle
Bisphenol A
Endocrine disrupting chemical
Radioactivity

ABSTRACT

A new system based on laccase-modified silica nanoparticles has been developed and tested for its ability to degrade a major endocrine disrupting chemical, 4,4′-isopropylidenediphenol (bisphenol A). The nanoparticles have been produced using the Stöber method and characterized using scanning electron microscopy, dynamic light scattering and ζ-potential measurements. The introduction of primary amino groups at the surface of these particles has been achieved using an organo-silane (amino-propyl-triethoxysilane). The use of glutaraldehyde as bi-functional coupling agent allowed the efficient conjugation of a laccase from *Coriolopsis polyzona* at the surface of the nanoparticles, as monitored by measuring the amount of proteins coupled and the ζ-potential of the produced nanoparticles. The oxidative activity of the so-produced bio-conjugate was tested using radioactive-(^{14}C) labeled bisphenol A. Analytical methods based on high performance liquid chromatography coupled to mass spectrometry and gas chromatography allowing a convenient and reliable study of the enzymatic activity of the produced bio-conjugates have been developed. It is demonstrated that even if a decrease of the specific catalytic activity of the immobilized enzyme is measured, the activity of the bio-conjugate remains compatible with the application of these systems to the transformation of phenolic pollutants. Additionally, the developed analytical methods allowed the identification of the transformation products formed during the enzymatic reaction.

1. Introduction

Bisphenol A (4,4′-isopropylidenediphenol, BPA) is a high production–volume chemical [1]; it is used mainly for the production of polycarbonates and epoxy resins. Additional applications of BPA include flame retardants, polyester, polyacrylate, polyetherimide and polysulphone polymer production, to name but a few. Because of this widespread use and the lack of appropriate methods in wastewater treatment plant scale for its elimination, relevant BPA environmental contaminations are frequently detected. Indeed, BPA is found at significant concentrations (5–200 ng/L) in surface, ground- and seawater. The first report on the toxic effects of BPA on animals has been published in 1993. In this study, it was demonstrated that during autoclaving, a substance able to bind rat endocrine receptors was released from laboratory polycarbonate flasks [2]. Liquid chromatography, mass spectrometry and nuclear magnetic resonance studies allowed assigning this activity to BPA released from the flasks. Since this study, a vast amount of research papers and review articles have been dedicated to the assessment of the adverse effects of BPA [3–6]. This endocrine disrupting chemical (EDC) is also suspected to have major toxic effects on human health inducing oncogenesis [7–12]. Efficient and applicable techniques for removing BPA in wastewater treatment processes remains a challenge of a high environmental and public health significance; new economically and environmentally relevant methods for water treatment are needed. One promising approach to face this challenge consists in the use of enzymatic systems able to degrade BPA into non-toxic or easy to remove products. Among the possible enzymes known to degrade BPA, laccases are attracting an increasing attention because of their high stability and the possibility to produce them in large-scale amounts [13–16]. To date, they undoubtedly represent one of the most promising biocatalysts for BPA elimination applications. Laccases are versatile oxidative enzymes that are used in a wide range of industrial processes in various fields including food, paper, textile, and cosmetic industries [17]. They are also used for environmental applications such as the treatment of contaminated effluents [18]. Laccases are non-heme metalloproteases capable of oxidizing numerous phenolic substrates such as the endocrine disrupting chemicals nonylphenol and triclosan and a wide range of xenobiotics belonging to pharmaceuticals and personal care products, pesticides, etc. [13]. Nonetheless, the application of enzymes in continuous systems such as wastewater treatment plants remains a challenge

* Corresponding author. Fax: +41 61 467 44 57.
E-mail address: patrick.shahgaldian@fhnw.ch (P. Shahgaldian).

0021-9797/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2010.05.031

as it is limited by the early washing out of the bio-catalysts under the hydraulic retention time conditions that are usually applied (<10 h). Recent efforts have been focused on the immobilization/encapsulation of bio-catalysts in order to tackle this major limitation and to facilitate their possible reuse. Among the different approaches tested, Cabana et al. have developed cross-linked enzyme (laccase) aggregates (CLEAs) from white rot fungal strain *Coriolopsis polyzona* and demonstrated that the developed systems possesses the ability to efficiently degrade nonylphenol, bisphenol A and triclosan in a fluidized bed [19] or in a perfusion basket reactor [20]. The same group reported successful immobilization of the same laccase on Celite® R-633 support and catalytic oxidative transformation activity of BPA has been demonstrated [21]. Based on these promising results, we have decided to develop a novel approach using silica nanoparticles as a carrier material to be modified with laccases. The choice of the carrier material has been motivated by the following factors: (i) big surface-to-volume ratio of the carrier that is expected to allow the immobilization of a large amount of enzyme to a relatively low amount of carrier material; (ii) the relative low cost of silica-based nano- and microparticles; (iii) abundance of silica in natural media. It is expected that the produced nanomaterials would have a relevant potential for a use is water purification plants.

In the present manuscript, we report on a new nanoparticulate system, based on silica nanoparticles chemically modified with a laccase from *C. polyzona* and its application to the degradation of BPA. Based on a new analytical procedure based on HPLC, LC-MS and GC-MC, we demonstrate that the produced nanoparticles are efficient for degrading BPA. The developed method, based on LC-MS(MS) and GC-MS allowed the identification of the transformation products formed during the enzymatic reaction.

2. Experimental procedure

2.1. General

Analytical grade chemicals were purchased from Fluka (Switzerland) and analytical grade solvents from J.T. Baker (Switzerland) and used without further purification. Radioactive ^{14}C-labeled BPA was purchased from Hartmann Analytic (Germany) with a specific radioactivity of 3.7×10^6 Bq mL^{-1} at 0.5 mM concentration in water. Laccase from *C. polyzona* was obtained from Wetlands Engineering (Belgium). Pure water (resistivity > 18 MΩ cm) was obtained using a Purelab® (Elga, France) water purification system.

2.2. Synthesis

2.2.1. Amino-functionalized nanoparticle synthesis

Silica nanoparticles were produced using the Stöber method [22]. Briefly, under moderate magnetic stirring, in 47 mL of ethanol were added 4 mL of tetraethyl orthosilicate (TEOS) and 3.3 mL of ammonium hydroxide (24% in water). The reaction mixture turned to a white milky suspension within few hours which was maintained under magnetic stirring at 20 °C during additional 24 h. The resulting suspension was transferred into a filtration stirring cell (Amicon, Milipore, Switzerland) equipped with a cellulose filtration membrane (cut-off 30 kDa) and thoroughly washed with pure water (2 L). To this suspension was added 300 μL of 3-aminopropyl-triethoxy-silane (APTES) and the reaction maintained under vigorous stirring during 20 h and consequently washed by centrifugation/re-suspension cycles (3000*g*, 3 min) using a Sörensen phosphate buffer (NaH_2PO_4: 30 mM, Na_2HPO_4: 40 mM; pH7); this step was repeated three times. The mass of the nanoparticles after each reaction step was estimated by replacing the buffer by pure water, centrifuging down 0.5 mL of the suspension and measuring the mass of the pellet dried under reduced pressure (20 mbar).

2.2.2. Enzyme-nanoparticle coupling

To a suspension of 200 mg of amino-functionalized nanoparticles in 9.75 mL of 67 mM Sörensen phosphate buffer (pH 7) was added 250 μL of glutaraldehyde (50% in water). The reaction mixture was maintained under magnetic stirring during 60 min. The produced glutaraldehyde-activated nanoparticles were washed by three centrifugation/re-suspension cycles (3000*g*, 3 min) using the same buffer. The laccase stock-solution was either directly diluted in a McIlvaine buffer (NaH_2PO_4: 90 mM, citric acid: 50 mM; pH 5) or dialyzed 24 h against the same buffer using a 20 kDa cut-off Float-A-Lyzer cellulose membrane set from Spectrum Labs (USA).

The total protein content of the stock solution was evaluated at 99 ± 2 mg mL^{-1} using the Lowry test [23] while the dialyzed solution showed a content of 63 ± 3 mg mL^{-1}. The enzymatic activity of the stock solution provided by the manufacturer was estimated at 1'620'000 U L^{-1} (measured with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), as a substrate in sodium-tartrate buffer (0.1 M, pH 4.5)), as described elsewhere [13]. One unit (U) was defined as the amount of enzyme forming 1 μmol of ABTS$^+$ per minute. A 22 mg of either the purified or the non-purified enzyme was incubated with 150 mg of the glutaraldehyde-activated nanoparticles at 4 °C during 12 h. The so-produced nanoparticles were washed three times by centrifugation-re-suspension. As most of the enzyme is getting released during the first washing step, the amount of bound-proteins was evaluated indirectly by measuring the quantity of enzyme remaining in the first supernatant using the Lowry test.

2.3. Nanoparticle characterization

2.3.1. Scanning electron microscopy (SEM)

Samples for SEM studies were prepared spreading 10 μL of the nanoparticle suspensions on freshly cleaved mica surfaces and dried at room temperature. After Au–Pd sputter coating, imaging was carried out using a (freshly calibrated using latex beads) Supra 40 V system (Carl Zeiss, Switzerland) at an accelerating voltage of 20 kV using an in-lens detector. Statistical size measurements were performed measuring the diameter of at least 200 nanoparticles using the analysis® (Olympus, Germany) software package.

2.3.2. Dynamic light scattering (DLS) and ζ-potential measurements

Particle size measurements were carried out by means of a Zetasizer nano-ZS (Malvern Instruments UK) on suspensions of the produced silica NPs in pure water. ζ-potentials were measured by electrophoretic mobility measurements with the same instrument and disposable capillary cells DTS 1060 (Malvern Instruments, UK). Both DLS and ζ-potential measurements were carried out in triplicate to ensure the reproducibility of the results.

2.4. Enzymatic transformation experiments

Laccase activity was tested monitoring the transformation of ^{14}C-labeled BPA. Diluted with non-labeled BPA, the assays were carried out in a total volume of 5 mL of McIlvaine buffer (0.1 M phosphate/0.05 M citric acid; pH 5) containing an absolute concentration of 1 mM of BPA (emitting a beta radiation signal of 33.3 kBq/mL). A 6.5×10^{-3} mg mL^{-1} of laccase were added and the reactions carried out at a temperature of 20 °C using increasing reaction times, and stopped by adding one volume equivalent of acetonitrile. Because of the formation of polymeric materials suspected to be caused mainly by the polymerization of the produced oxidation products [24,25], the solutions were filtered using a ny-