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A selective and sensitive D-xylose electrochemical biosensor based on xylose dehydrogenase displayed on the surface of bacteria and multi-walled carbon nanotubes modified electrode

Liang Li[a,b,1], Bo Liang[a,1], Jianguo Shi[c], Feng Li[b], Marco Mascini[d,**], Aihua Liu[a,*]

[a] Laboratory for Biosensing, Qingdao Institute of Bioenergy & Bioprocess Technology, and Key Laboratory of Bioenergy, Chinese Academy of Sciences, 189 Songling Road, Qingdao 266101, China
[b] College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, 53 Zhengzhou Road, Qingdao 266042, China
[c] Key Laboratory for Biosensors of Shangdong Province, Biology Institute of Shandong Academy of Sciences, 19 Keyuan Road, Jinan 250014, China
[d] Dipartimento di Chimica, Universita degli Studi di Firenze, Via della Lastruccia, 3 50019 Sesto Fiorentino, Italy

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ABSTRACT

A novel Nafion/bacteria-displaying xylose dehydrogenase (XDH)/multi-walled carbon nanotubes (MWNTs) composite film-modified electrode was fabricated and applied for the sensitive and selective determination of D-xylose (INS 967), where the XDH-displayed bacteria (XDH-bacteria) was prepared using a newly identified ice nucleation protein from *Pseudomonas borealis* DL7 as an anchoring motif. The XDH-displayed bacteria can be used directly, eliminating further enzyme-extraction and purification, thus greatly improved the stability of the enzyme. The optimal conditions for the construction of biosensor were established: homogeneous Nafion–MWNTs composite dispersion (10 μL) was cast onto the inverted glassy carbon electrode, followed by casting 10-μL of XDH-bacteria aqueous solution to stand overnight to dry, then a 5-μL of Nafion solution (0.05 wt%) is syringed to the electrode surface. The bacteria-displaying XDH could catalyze the oxidization of xylose to xylonolactone with coenzyme NAD^+ in 0.1 M PBS buffer (pH7.4), where NAD^+ (nicotinamide adenine dinucleotide) is reduced to NADH (the reduced form of nicotinamide adenine dinucleotide). The resultant NADH is further electrocatalytically oxidized by MWNTs on the electrode, resulting in an obvious oxidation peak around 0.50 V (vs. Ag/AgCl). In contrast, the bacteria-XDH-only modified electrode showed oxidation peak at higher potential of 0.7 V and less sensitivity. Therefore, the electrode/MWNTs/bacteria-XDH/Nafion exhibited good analytical performance such as long-term stability, a wide dynamic range of 0.6–100 μM and a low detection limit of 0.5 μM D-xylose (S/N = 3). No interference was observed in the presence of 300-fold excess of other saccharides including D-glucose, D-fructose, D-maltose, D-galactose, D-mannose, D-sucrose, and D-cellbiose as well as 60-fold excess of L-arabinose. The proposed microbial biosensor is stable, specific, sensitive, reproducible, simple, rapid and cost-effective, which holds great potential in real applications.

1. Introduction

In nature, carbohydrates can be involved in many important life activities to provide carbon resource and energy (Beckham et al., 2011). D-Xylose (INS 967) has been recognized as the ideal sweetener, nutritional agents and therapeutic agents for diabetics since 1960s (Broekaert et al., 2011). Xylose-related products such as xylan and xylo-oligosaccharides also have a high functional value, which have been widely applied to the efficacy of food and other health effects of food. Additionally, xylose can be used as softener, surfactant, and plasticizer in various industries (Martel et al., 2010). On the other hand, xylose is one of the main hydrolysis products of cellulose; the fermentation of xylose could enhance the utilization efficiency and the yields of fuel ethanol (Elkins et al., 2010). Thus, both fermentability and conversion efficiency of xylose are important factors in identifying superior engineering strains and scaling-up production in bioenergy industry (Lopez-Casado et al., 2008). Therefore, the xylose analysis in food, medicine and biological processes is extremely important. Traditional methods to detect sugars include reduction, chromatography, refractive index, UV and calorimetric method, Raman spectroscopy (Shih et al., 2011) and near Infrared spectroscopy (Morita et al., 2011), although these methods of analysis and detection of various sugars have reached

* Corresponding author. Tel.: +86 532 8066 2758.
** Corresponding author.
E-mail addresses: marco.mascini@unifi.it (M. Mascini), liuah@qibebt.ac.cn (A. Liu).
[1] These two authors contributed equally to this work.

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a high degree of separation and sensitivity level, they still have a wide variety of application constraints. As the conventional method of sugar analysis, reduction method for reducing sugar, which is a feature that can be used Fehling method and 3,5-dinitrosalicylic acid method to determine xylose. However, the Fehling method lost selectivity and was difficult to judge the end-point when the xylose content is less than 0.3%. High-performance liquid chromatography (HPLC) (Cheng et al., 2010; He et al., 2003; Rovio et al., 2008; Sharma et al., 2010; Wan and Yu, 2007) and capillary electrophoresis methods (Grossl et al., 2005; Huang et al., 2005) are ideal for sugar analysis, nevertheless, which have disadvantages such as expensive reagents required in sample preparation, long analysis time and low resolution.

Electrochemical methods were also reported for the direct detection of xylose: for instance, a pulsed amperometric detection on an electrophoretic microchip (Fanguy and Henry, 2002) and potentiometric biosensor based on field effect transistor utilizing *Gluconobacter oxydan* whole cells (Reshetilov et al., 1996), however, the detection sensitivity is not satisfactory. Due to their high selectivity and sensitivity, electrochemical biosensors are useful for real-time monitoring of the target molecules in a more appropriate device. The biosensor is constructed by immobilization of enzyme onto the electrode surface, which can be realized to detect carbohydrates rapidly, sensitively, and selectively. To date, in combination with HPLC, many enzyme-based biosensing systems, for example, cellobiose dehydrogenase (Elmgren et al., 1992, 1993a,b; Nordling et al., 1993), glucose dehydrogenase (Marko-Varga, 1987), aldose dehydrogenase (Smolander et al., 1993, 1995), hexose oxidase (Maes and Nagels, 1993), pyranose oxidase and oligosaccharide glucose dehydrogenase (Ikeda et al., 1989, 1990; Kiba et al., 1991; Kinoshita et al., 1990; Ruzgas et al., 1996; Tessema et al., 1995) have been developed for the measurement of sugars, nevertheless, these approaches are still time-consuming, expensive, and complex in procedure. In most cases, the enzyme-based sensors were actually used as the electrochemical detectors after HPLC separation. Further, few reports could be found with xylose dehydrogenase (XDH) system for xylose detection. So it is high desire to establish method which is rapid, simple in instrumentation and operation, sensitive, selective and cost-effective.

The rapid development of nanotechnology provides a strong strategy for analytical chemistry, which helps people to understand the nano-bio-interface interaction in depth (Höök et al., 2008; Liu, 2008; Liu et al., 2006b). Nano-structured materials, due to their excellent chemical properties, have been widely used to explore sensitive detection system (Rickerby and Morrison, 2007). For example, carbon nanotubes (CNTs) are ideal materials for the nanoelectronic devices and nano-sensors because of CNTs' unique electronic properties, large surface area, excellent electrochemical performance and good chemical stability (Andrews et al., 2002; Ouyang et al., 2002). Actually, CNTs can facilitate electron transfer reaction (Baughman et al., 1999) and increase the catalytic activity (Banks et al., 2004).

$$\text{Xylose} + \text{NAD}^{+} \xrightarrow{\text{XDH}} \text{xylonolactonc} + \text{NADH} \quad (1)$$

XDH (EC 1.1.1.175) can catalyze D-xylose metabolism, which occurs through a contrasting pathway that differs from the cases for classical D-xylose pathway in most bacteria (Buchert and Viikari, 1988; Suzuki and Onishi, 1973; Yamanaka et al., 1977). XDH could convert D-xylose to D-xylonolactone with NAD^+ (nicotinamide adenine dinucleotide) as its coenzyme, which is reduced to NADH (the reduced form of nicotinamide adenine dinucleotide) as shown in Eq. (1). As an outer membrane protein, ice-nucleation protein has been found in some prokaryote. It is anchored on the surface of bacteria by a glycosylphosphatidylinositol anchor, which benefits the surface display of proteins (Kawahara, 2002; van Bloois et al., 2011). XDH gene had been cloned from the genome of strain *Caulobacter crescentus* NA1000 due to its high selectivity and enzyme activity (Stephens et al., 2007). N terminus gene of ice-nucleation protein had been cloned from the genome of strain *Pseudomonas borealis* DL7 (Wu et al., 2009). Inspired by this idea, a recombinant plasmid pTInaPb-N/Xdh was constructed in the way that XDH gene was fused into N terminus gene of ice-nucleation protein in our laboratory (Liang et al., 2012). The plasmid could be digested into a 1300 bp strip by *Nco* I/*Hind* III double enzyme. Recently, the XDH cell-surface displaying system using a newly identified ice nucleation protein from *P. borealis* DL7 as an anchoring motif was developed in our laboratory (Liang et al., 2012). Considering that the practical applications of xylose detection by intracellular protein could be limited by the cost of purification and the stability of the free enzyme, the strategy of bacteria surface expression of XDH may be an ideal method for sensing application.

In this paper, we constructed a novel D-xylose electrochemical biosensor using XDH-displayed bacteria (XDH-bacteria) combination of multi-walled carbon nanotubes. The proposed microbial biosensor is capable of high specificity, high sensitivity, improved stability, rapidness and low-cost. To the best of authors' knowledge, this is the first report to address combination of bacteria-surface-expression-of-enzyme with nanotechnology for sensitive and selective electrochemical biosensor application.

2. Materials and methods

2.1. Chemicals and reagents

Nafion (perfluorinated ion-exchange resin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich and used as received. 0.05 wt% Nafion solution was prepared from 5 wt% Nafion. Multi-walled carbon nanotubes (MWNTs) were kindly gifted from Prof. Dr. Gebo Pan in Suzhou Institute of Nan-Tech and Nano-Biomics, Chinese Academy of Sciences. All other reagents were of the highest grade available and used without further purification. All solutions were prepared with Milli-Q water.

2.2. Bacterial strains and plasmids, growth of bacteria-displayed XDH

The details for the construction of plasmid pTInaPbN/Xdh and the growth of bacteria-displayed XDH can be found elsewhere (Liang et al., 2012). Briefly, *Escherichia coli* BL21 (DE3) expressing XDH on the cell surface was used. Plasmid pTInaPbN/Xdh was used to express InaPb-N/XDH on the cell surface. Strains harboring the expressing vectors pTInaPb-N/Xdh were grown at 37 °C in Luria–Bertani (LB) medium supplemented with kanamycin to a final concentration of 30 μg/mL with stirring at 200 rpm. When OD_{600} value of bacterial was up to 0.5, they were induced with isopropyl-β-D-thiogalactopyranoside (IPTG, 0.5 mM) at 25 °C. After 24 h of growth, the harvested cells were washed and diluted to unit cell density (OD_{600} = 1.0) with 50 mM PBS buffer (pH 8.0) and stored at 4 °C. The concentration of the XDH-displayed *E. coli* is assayed to be 1.4×10^8 cfu/mL. The XDH activity of the whole cell reached to 1.89 ± 0.03 U/OD_{600} after the enzyme expression induced by IPTG (one unit of enzyme activity is 1 μmol NADH generated per min per OD_{600} whole cells.), suggesting that XDH was successfully displayed on the surface of ice-nucleation protein bacteria.