Biosensors and Bioelectronics 62 (2014) 151–157

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Development of spiropyran-based electrochemical sensor *via* simultaneous photochemical and target-activatable electron transfer

Jia Tao [1], Yinhui Li [1], Peng Zhao, Jishan Li, Yu Duan, Wenjie Zhao, Ronghua Yang *

*State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, and Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha 410082, China*

ARTICLE INFO

*Article history:*
Received 20 March 2014
Received in revised form
22 May 2014
Accepted 24 May 2014
Available online 11 June 2014

*Keywords:*
β-galactosidase
Electrochemistry detection
Spiropyran
Photo-activation
Target-activation

ABSTRACT

In traditional electrochemical sensors, the electrochemical signal transduction of the redox-active material is usually controlled by the analytical target. Due to *non*-specific interaction between the redox mediator and the target, false signal by single stimulus may not be avoided. To address this issue, we have developed a new electrochemical sensor that uses a functional spiropyran, an important class of photo and thermochromic compounds, as both recognition receptor and latent redox mediator, to realize simultaneous photochemical and target-modulated electron transfer. As a proof of principle, β-galactosidase was chosen as a model target. The new synthesized spiropyran probe, SP-β-gal, undergoes reversibly structural isomerization to form merocyanine under UV light irradiation. After the glycosidic bond being cleaved by β-galactosidase, the opened merocyanine of SP-β-gal forms redox-active 2-(2.5-dihydroxystyryl)-1.3.3-trimethyl-3H-indolium, and thus produces a pair of reversible redox current peaks under the electrochemical scanning. To amplify the detection signal, SP-β-gal was self-assembled with single-walled carbon nanotubes (SWCNTs) on the surface of glass carbon electrode. Kinetics experiments confirm that the probe is an ideal candidate for the determination of different concentrations of β-galactosidase digestion kinetics. Further, the SP-β-gal/SWCNTs-modified electrode is chemically stable in complex biological fluids. It was successfully applied to monitor β-galactosidase activity in the 10% calf thymus. This work represents not only a significant step forward in the further development of low-dimensional carbon nanomaterials/small organic molecular probes-based electrochemical biosensors, but also a new platform which may be extended to the assay of other enzyme such as β-D-glycosidase and so on by translating the biorecognition into electrochemical signal responses.

© 2014 Published by Elsevier B.V.

## 1. Introduction

The monitoring of important chemical and physiological active substance needs quick, sensitive, real-time and on-line detection technology. Usually, electrochemical sensors are considered to be one of the most hopeful detection tools that could meet the need of life physical activity research (Privett et al., 2010; Kimmel et al., 2012). To design an electrochemical sensor, the decorating materials and molecular recognition elements modified on the electrode's interface are the most important (Gooding, 2005), making the exploration of electrically active materials and fabrication of the electrode's interface widely reached in electrochemical biosensings (Hartwell and Grudpan, 2010; Jacobs et al., 2010). Traditionally, electrochemical sensors utilize natural or artificial redox mediators to monitor a reaction of an electrode, and the electrochemical response is controlled by an analytical target (Gooding, 2005). Due to the *non*-specific interaction between the redox mediator and the target, false electrochemical signal based on singled stimulus may not be avoided (Fan et al., 2003; Wang et al., 2009). Thus, it is necessary to produce multi- stimuli modulation to compensate for these effects. A special kind of materials called photoswitchable biomaterials is available in the electrochemistry to generate discrete "On" and "Off" states in response to the changes in the ambient light (Willner and Rubin, 1996). These materials isomerize reversibly upon light irradiation, and the discrete photoisomeric states exhibit distinct spectral and chemical features (Willner and Rubin, 1996; Lion-Dagan et al., 1995; Willner et al., 1995, 1991a,b). The major challenge to carry out a light and target-double modulated signal transduction of an electrochemical sensor is to create a photoswitchable redox mediator exhibiting specific recognition properties for the substrate over other interferences.

Spiropyrans, an important class of photochromic compounds, undergo reversibly structural isomerization between ring-closed spiropyran form and ring-opened merocyanine (Fischer and

* Corresponding author. Tel./fax: +86 731 88822523.
*E-mail address:* Yangrh@pku.edu.cn (R. Yang).
[1] Co- first authors: These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2014.05.072
0956-5663/© 2014 Published by Elsevier B.V.

Hirshberg, 1952). Pioneered by the seminal work of Willner et al. of photostimulation of biomacromolecules with photochromic polymers (Willner et al., 1991a,b), spiropyran derivatives have been utilized for the electrochemical sensors by regulating the electron transfer between the electrode surface and the targets (Willner et al., 1994; Preigh et al., 1996; Blonder et al., 1998; Guo et al., 2004a,b; Wang et al., 2006; Wagner et al., 2011). However, in those systems, the spiropyrans were functionalized as the photoswitchable modulator to trigger electron transfer. The electrochemical assays based on the spiropyrans are limited to redox-active targets (Preigh et al., 1996; Guo et al., 2004a,b; Wagner et al., 2011). For detections of *non*-redox targets, additional redox-active materials are needed to transduce an electrochemical signal (Willner et al., 1994; Blonder et al., 1998; Wang et al., 2006). But the use of inherent redox mediator in the electrochemical cell may lead to the appearance of instability and false signals. To address this issue and as a part of our ongoing interest in spiropyran sensor designs (Shao et al., 2006, 2010; Li et al., 2012), we expect to construct a new spiropyran-based electrochemical sensor that uses spiropyran as a latent redox mediator to be activated by light and the target. This approach not only rules out the need of inherent redox mediator existing in the electrochemical cell for detection of *non*-redox targets, but also improves the signal veracity *via* simultaneous photochemical and target-activated electron transfer. To the best our knowledge, no such a spiropyran-based electrochemical sensor has been reported in the literature.

As a proof of principle, we utilized β-galactosidase as a model target. Glycosidase, also called glycoside hydrolase, is a very important class of hydrolytic enzyme and play a central role in many organisms (Yang et al., 2013; Choi et al., 2013). The glycosidase level and its activity in the living systems have a close relationship with various diseases, for example diabetes. The increasing understanding of the relationship between β-galactosidase and various diseases promotes the development of new β-galactosidase sensing strategies. For this purpose, several colorimetric and fluorescent probes have been developed for analysis of β-galactosidase by now (Zeng et al., 2012; Li et al., 2011; Koide et al., 2009; Komatsu et al., 2006). However, most probes suffer from disadvantages in complex biosystem, such as the poor cellular permeability, photobleaching, background fluorescence and so on. To solve this problem, herein, we designed and synthesized a new electrochemical redox mediator, SP-β-gal, for β-galactosidase detection. The probe exhibits simultaneous photochemical and target-modulated electron transfer. It could effectively avoid the false signal caused by other components. Moreover, the electrochemical method could satisfy the need of sensitivity in real samples. The enzyme recognition scheme is derived from a specific hydrolysis reaction of the glycosidic bond. The spiropyran compound bears one hydroxyl spiropyran backbone which can universally form a merocyanine structure with UV light irradiation. On the other hand, because the glycosidic bond of β-galactose exhibits high affinity for β-galactosidase in aqueous solution, we expect that the hydrolyzation of SP-β-gal by β-galactosidase would cooperatively create a hydroquinone-appended spiropyran molecule giving a new electrochemistry response. To establish an efficient electrode's interfaces and amplify electrochemical signal, single-walled carbon nanotubes (SWCNTs) modified glass carbon electrode was used as the electrochemical device, because SWCNTs have the ability to promote electron transfer reactions, and a high aspect ratio to increase the electro-active surface area when modified onto the working electrode (Hu and Dong, 2008; Zhao et al., 2002). More importantly, the strong affinity of SWCNTs with hydrophobic organic molecules will facilely absorb functionalized spiropyran on the electrode surface by π–π stacking interaction (Tournus et al., 2005; Hrapovic et al., 2004).

## 2. Experimental section

### 2.1. Materials and apparatus

β-galactosidase from *Escherichia coli* was purchased from Sigma-Aldrich. All the solvents and other chemicals were of analytical reagent grade and were supplied by Alfa Aesar or Sigma-Aldrich. The carboxylated SWCNTs were purchased from Carbon Nanotechnologies, Inc., Houston, TX. For acid-based oxidation of SWCNTs, 300 mg of SWCNTs were treated with 60 mL of 30% nitric acid solution and was sonicated in water bath sonicator at ambient condition for 2 h.

Cyclic voltammetry (CV) was performed with a three electrode cell linked to a CHI instruments model 600D electrochemical analyzer (Shanghai, China). The electrochemical cell consists of a Pt wire counter electrode, an Ag quasi-reference electrode (QRE), and a glass carbon electrode (GCE) as working electrode (WE). Transmission electron microscopy (TEM) measurements were conducted on a JEOL 1230 electron microscope. The UV absorption spectrum was measured using a U-4100 HITACHI spectrophotometer. Atomic force microscope (AFM) was conducted on a SPI3800-SPA400 microscope. Zeta potential measurement was used to monitor the surface potential of the SWCNTs with a Zetasizer Nano ZS90 (Malvern Instruments, U.K.). Mass spectrometry (MS) was conducted on an MAT 95 XP produced by Thermo Finnigan (USA). Nuclear magnetic resonance (NMR) was conducted on an INOVA-400 produced by Varian (USA).

### 2.2. Synthesis of SP-β-gal

The routs for the synthesis of SP-β-gal were shown in Scheme S1. For preparation of **3**, into a 50 mL round-bottomed flask equipped with a magnetic stirring bar containing 1, 3, 3-trimethyl-2-methyleneindoline (0.86 g, 5 mmol) in a 10 mL dry THF solution, 2, 5-dihydroxybenzaldehyde (0.83 g, 6 mmol) was added. The mixture was heated to reflux for 12 h under an argon atmosphere and then was cooled to room temperature. The solvent was removed under reduced pressure. Remaining purple solid was subsequently recrystallized from a mixture of hexane/chloroform to give a needle-like light brownish crystal (1.17 g, 80%).

*Preparation of* **5.** The solution of **3** (0.88 g, 3 mmol) in acetone (10 mL) was treated with sodium hydroxide solution (1.00 M, 3 mL). **4** (1.64 g, 4 mmol) in acetone (10 mL) was added dropwise with stirring over 30 min. After stirring for 10 h in the dark, TLC showed complete consumption of the galactosyl bromide and some of **3** remained unreacted. A second portion of 2,3,4,6 tetra-O-acetyl-β-galactopyranosyl bromide (0.82 g; 2 mmol) was added, together with an equivalent of sodium hydroxide (1.00 M, 2 mL). The solution was stirred a further 10 h, after which time the reaction was complete. The acetone was evaporated and the residue redissolved in chloroform (50 mL), which was extracted exhaustively with dilute potassium carbonate solution, washed, and dried ($MgSO_4$). After furified by flash chromatography, 1.42 g (76%) compound **5** was obtained.

*Preparation of SP-β-gal*. A solution of 5 (1.28 g; 2 mmol) in warm, dry methanol (20 mL) was treated with methanolic sodium methoxide (0.5 mL). After 1 h, the solvent was evaporated. Then, the resulting mixture was poured into a mixture of chloroform and water (300 mL of each). The organic phase was separated and the aqueous phase was extracted three times with chloroform. The combined organic extracts were washed with water, saturated

Download English Version:

https://daneshyari.com/en/article/7233001

Download Persian Version:

https://daneshyari.com/article/7233001

Daneshyari.com