Biosensors and Bioelectronics 105 (2018) 129–136

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Label-free electrochemical biosensors based on 3,3′,5,5′-tetramethylbenzidine responsive isoporous silica-micelle membrane

Qinqin Sun, Fei Yan, Bin Su*

Institute of Analytical Chemistry, Department of Chemistry, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Keywords:
Label-free
DNAzyme
TMB
Electrochemical detection
Silica-micelle membrane

ABSTRACT

3,3′,5,5′-Tetramethylbenzidine (TMB) has been frequently used as an indicator in G-quadruplex/hemin DNAzyme (G4zyme)-based chemical and biochemical analysis, and its oxidation products are usually monitored by electrochemical or optical methods to quantify G4zyme formation-related analytes. Herein we report a simple electrochemical approach based on isoporous silica-micelle membrane (iSMM) to measure TMB, instead of its oxidation products, in G4zyme-based detection of specific analytes. The iSMM was grown on the indium tin oxide (ITO) electrode, which was composed of highly ordered, vertically oriented silica nanochannels and cylindrical micelles of cetyltrimethylammonium. The iSMM-ITO electrode was selectively responsive to neutral TMB but not its oxidation products, thanks to the sieving and pre-concentration capacity of micellar structures in terms of molecular charge and lipophilicity. In other words, only TMB could be extracted and enriched into micelles and subsequently oxidized at the underlying ITO electrode surface (namely the micelle/ITO interface), generating an amplified anodic current. Since the depletion of TMB was catalyzed by G4zymes formed in the presence of specific analyte, the decrease of this anodic current enabled the quantitative detection of this analyte. The current variation relative to its initial value ($(j_0-j)/j_0$), termed as the current attenuation ratio, showed the obvious dependence on the analyte concentration. As proof-of-concept experiments, four substances, i.e., potassium cation (K^+), adenosine triphosphate, thrombin and nucleic acid, were detected in aqueous media and the analysis of K^+ in pre-treated human serum was also performed.

1. Introduction

The depletion of 3,3′,5,5′-tetramethylbenzidine (TMB) has been frequently used in horseradish peroxidase (HRP) and G-quadruplex/hemin DNAzyme (G4zyme)-based chemical and biochemical analysis (Josephy et al., 1982; Li et al., 2009a). G4zymes are complexes of hemin and guanine-rich (G-rich) nucleic acid segments, which possess HRP-mimicking activity and have been successfully employed as the signal transduction elements in numerous label-free biosensors. In comparison with HRP, its advantages include superior chemical/thermal stability, low cost, tolerance of long-term storage and diverse modifications (Li et al., 2009a, 2009b, 1996; Travascio et al., 1998). In a popular sensing scheme, G4zyme catalyzes the oxidation of TMB by H_2O_2 and the depletion of TMB reflects the catalytic activity of G4zyme. Thus, by recording the depletion of TMB, one is able to quantify the G4zyme and any building units (usually the target analyte) of the G4zyme. Based on this scenario, a large number of well-designed analytical strategies have been reported so far for the quantitative detection of ions (Li et al., 2009b; Wu et al., 2016; Yang et al., 2010; Yin et al., 2009), small molecules (Du et al., 2011; Kong et al., 2010; Wang et al., 2014b; Wu et al., 2016; Yang et al., 2012; Zhang et al., 2015), proteins (Freeman et al., 2010; Liu et al., 2013; Zhang et al., 2011), and nucleic acids (Alves-Balvedi et al., 2016; Chen et al., 2011; Lin et al., 2016; Shi et al., 2015; Yu et al., 2015; Zhang et al., 2011; Zhang et al., 2014; Zhu et al., 2011).

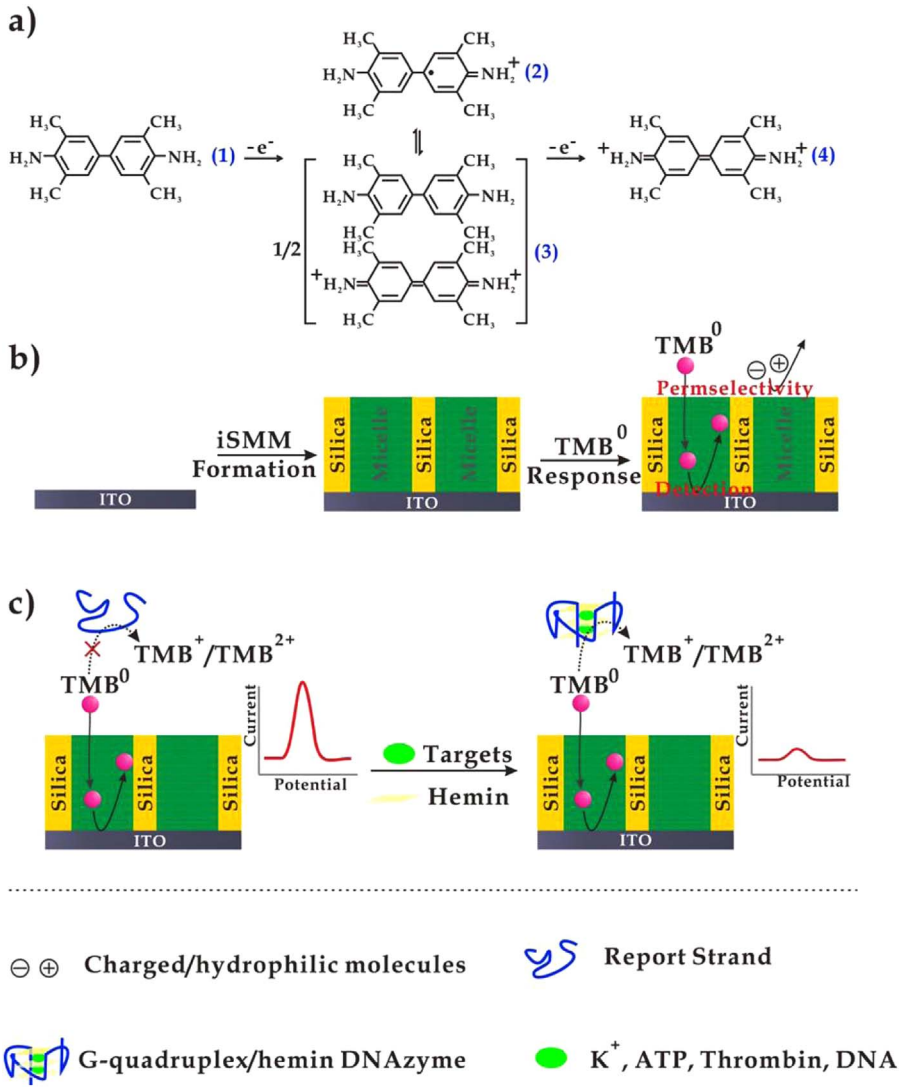
In most biosensors, the depletion of TMB is monitored by the generation of its oxidation products, instead of measuring the quantity of TMB directly. Under the catalysis of G4zyme, TMB (namely **1** in Scheme 1a) undergoes two sequential single-electron oxidation steps to yield cationic radical (namely **2**), charge-transfer complex (CTC) (namely **3**) and diimine (namely **4**) (Josephy et al., 1982). The detection of oxidation products can be accomplished by the UV–vis absorption spectroscopy (Du et al., 2011; Wang et al., 2014a, 2014b; Wu et al., 2016; Yang et al., 2012, 2010; Yin et al., 2009; Zhang et al., 2015, 2011; Zhu et al., 2011), Raman (Laing et al., 2011), quartz crystal microbalance (Martin et al., 2002), and electrochemistry (Alves-Balvedi et al., 2016; Baldrich et al., 2009; Cai et al., 2013; Chen et al., 2011; Fanjul-Bolado et al., 2005; Sun et al., 2013; Volpe et al., 1998; Wan et al., 2013; Wang

* Corresponding author.
E-mail address: subin@zju.edu.cn (B. Su).

https://doi.org/10.1016/j.bios.2018.01.026
Received 16 September 2017; Received in revised form 21 December 2017; Accepted 12 January 2018
Available online 31 January 2018
0956-5663/

et al., 2014a; Yu et al., 2015; Zhang et al., 2014). Among these, UV–vis absorption spectroscopy is most frequently used, in which the absorbance of either one-electron oxidation product (namely CTC, **3**) at 652 nm or two-electron oxidation product (namely diimine, **4**) at 450 nm is measured. However, the relative quantities of oxidation products at two charge states are dynamic, considering the gradual conversion of **2** and **3** to **4** in the presence of excess H_2O_2. Hence one additional treatment is often done by adding strong acids, such as HCl or H_2SO_4, to facilitate the conversion of **2** and **3** to **4** (Du et al., 2011; Li et al., 2009a, 2009b; Wang et al., 2014b; Wu et al., 2016; Yin et al., 2009; Zhang et al., 2011, 2007; Zhu et al., 2011). However, the strong acid may cause irreversible damage to biological samples in some cases.

elements on the sensor surface and subsequently tether of signal transduction element (G4zyme or HRP). This approach usually suffers from the time-consuming and labor-intensive electrode pretreatment, multiple steps of immobilization and chemical modification, low reactivity, and complicated operation (Zhang et al., 2016). In this case, electrochemical biosensors are becoming more and more advantageous. For instance, Wang et al. proposed a polymeric liquid membrane electrode functionalized by an appropriate lipophilic cationic exchanger to quantify the cationic TMB oxidation products by potentiometry (Wang et al., 2014a). The method has been successfully used in HRP and G4zyme-based biosensing with a high analytical sensitivity. However, to the best of our knowledge, electrochemical sensors that



Scheme 1. (a) Schematic Illustration of Successive Oxidation of TMB (**1**) to Cationic Radical (**2**), Charge-Transfer Complex (**3**) and Diimine (**4**) in Aqueous Solutions of pH = 5.10. (b-c) Schematic Principle of Label-Free Electrochemical Biosensors by Coupling (b) TMB Responsive iSMM-ITO and (c) the Analyte Modulated G-quadruplex/hemin DNAzyme Catalytic Activity towards TMB Depletion.

Thanks to attractive features of portability, low-cost, ease of miniaturization and integration, fast response and excellent resistance to color and turbid interferences, electrochemical sensors have found a broad range of applications in diverse fields (Zhang et al., 2016). Based on the redox properties of TMB oxidation products, many electrochemical biosensors have been developed to detect the activity of G4zyme or HRP and further probe the recognized target analytes (Baldrich et al., 2009; Chen et al., 2011; Fanjul-Bolado et al., 2005; Sun et al., 2013; Volpe et al., 1998; Wan et al., 2013; Yu et al., 2015; Zhang et al., 2014). However, most electrochemical sensors are fabricated in a heterogeneous mode, involving the immobilization of recognition

enable the analysis of G4zyme activity by direct quantifying TMB instead of its oxidation products has not been reported. It might be ascribed to the poor solubility of TMB in aqueous solutions (Frey et al., 2000), as well as its poor electrochemical response at conventional electrodes.

Herein we report a simple, label-free electrochemical approach that is capable of measuring directly TMB with the aid of isoporous silica-micelle membrane (iSMM). The iSMM primarily grown on the surface of indium tin oxide (ITO) electrodes (designated as iSMM-ITO electrodes) resembles a binary assembly of highly ordered, vertically aligned silica nanochannels and cylindrical surfactant micelles of