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Target-responsive aptamer release from manganese dioxide nanosheets for electrochemical sensing of cocaine with target recycling amplification

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ABSTRACT

A novel electrochemical sensing platform based on manganese dioxide (MnO_2) nanosheets was developed for sensitive screening of target cocaine with the signal amplification. Ferrocene-labeled cocaine aptamers were initially immobilized onto MnO_2 nanosheets-modified screen-printed carbon electrode because of π-stacking interaction between nucleobases and nanosheets. The immobilized ferrocene-aptamer activated the electrical contact with the electrode, thereby resulting in the sensor circuit to switch on. Upon target cocaine introduction, the analyte reacted with the aptamer and caused the dissociation of ferrocene-aptamer from the electrode, thus giving rise to the detection circuit to switch off. The released aptamer was cleaved by DNase I with target recycling. Under optimal conditions, the decreasing percentage of the electronic signal relative to background current increased with the increasing cocaine concentration in the dynamic range of 0.1–20 nM, and the detection limit was 32 pM. The reproducibility, selectivity and method accuracy were acceptable. Importantly, this concept offers promise for rapid, simple, and cost-effective analysis of cocaine biological samples without the needs of sample separation and multiple washing steps.

1. Introduction

A simple, sensitive and specific analytical method for highly efficient detecting and quantifying small molecules is very important for affordable medical diagnostics and toxin monitoring at home in modern healthcare [1]. Ongoing efforts have been made worldwide in the field of assay development to simplify the assay process with the aim of manufacturing portable and affordable diagnostic devices while preserving the essential benefits like sensitivity, robustness and broad applicability [2,3]. Generally speaking, the assay is executed by using certain affinity ligands, *e.g.*, antibody or aptamer, which specifically reacted with biomolecules to mediate a target-responsive signal transduction cascade [4]. Aptamers are single-stranded DNA or RNA oligonucleotides that have high affinity and specificity to their targets, which are generated by an *in vitro* evolution process called systematic evolution of ligands by exponential enrichment (SELEX) [5]. Upon binding to targets, aptamers usually have their conformations significantly changed into the hairpins, stem-loops, G-quadruplexes, pseudoknots or bulge structures [6–8]. Interest in nanomaterials with aptamers has increased rapidly in recent years as a result of their size- and shape-depended properties [9]. One of extremely useful advantages using nanostructures (*e.g.*, graphene oxide nanosheets) is that they protect aptamers from nuclease cleave owing to the steric-hindrance effect after being bond to the nanomaterials [10].

Manganese dioxide is potentially interesting pseudocapacitive material due to natural abundance, low cost, environmentally benign nature and high theoretical specific capacitance [11]. Nevertheless, the poor electrical electricity (10^{-5}–10^{-6} S cm^{-1}) and slow ion transport rate of MnO_2 limit its practical application in the electrochemical fields [12]. To improve this concern, it is important to design size- and shape-depended MnO_2 nanostructures with high surface coverage and good electricity conductivity [12]. Inspiringly, the rapidly emerging research filed of two-dimensional nanomaterials provides excitingly new possibilities for advanced development of new analytical tools and instrumentation for different applications [13]. Zhu et al. [14] synthesized MnO_2 nanosheets-covered submicrometer tube forests as binder-free electrodes for high energy density all-solid-state supercapacitors. Yu et al. [15] directly grew ultrathin MnO_2 nanosheets on conductive carbon fibers for the development of high-

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performance fiber-shaped all-solid-state asymmetric supercapacitors. Peng et al. [16] prepared MnO_2 nanosheet suspension for the adsorption of Cadmium(II) contamination in water body. He et al. [17] developed a sensitive turn-on fluorescent sensor based on single-layer MnO_2 nanosheet-quenched fluorescent carbon quantum dots for rapid and selective sensing of glutathione. Inspired by these examples, our motivation in this work is exploring a novel electrochemical sensing platform for the sensitive monitoring of small molecules (cocaine used in this case) coupling with aptamer and DNase I-based target recycling amplification strategy on MnO_2 nanosheets-modified interface.

Cocaine, a strong stimulant mostly used as a recreational drug, has a small number of accepted medical uses, and high doses can result in very high blood pressure or body temperature [18]. Also, cocaine is addictive because of its effect on the reward pathway in the brain. According to a 2007 United Nations report, Spain is the country with the highest rate of cocaine usage (3.0% of adults), while other countries are the United States (2.8%), England and Wales (2.4%), Canada (2.3%), Italy (2.1%) and Scotland (1.5%) [19]. Herein we report the proof-of-concept of simple and powerful electrochemical detection system for target cocaine by immobilizing ferrocene-labeled aptamer onto MnO_2 nanosheets-functionalized screen-printed carbon electrode (Scheme 1). The electrochemical signal originates from the labeled ferrocene, which is amplified by DNase I-based catalytic cleavage accompanying target recycling. Initially, ferrocene-aptamer conjugates are adsorbed onto MnO_2 nanosheets-modified electrode *via* π-stacking interaction between nucleobases and nanosheets. Introduction of MnO_2 nanosheets can efficiently protect the aptamers from nuclease cleavage. Upon addition of target cocaine, the analyte reacts with the aptamer and disturbs the interaction between ferrocene-aptamer and the nanosheets, therefore causing the dissociation of the conjugate from the electrode. Meanwhile, the released cocaine/aptamer-ferrocene complex is cleaved by DNase I, and target cocaine is delivered from the complex, which re-attacks other ferrocene-aptamer conjugates on the electrode with target recycling. In this case, numerous ferrocene-aptamer conjugates are liberated from the electrode, thus resulting in the decreasing electronic signal. By monitoring the shift in the current, we quantitatively evaluate the cocaine. Just as the protection of MnO_2 nanosheets toward aptamers and DNase I-based target recycling, our method is expected to quantify cocaine with high sensitivity and specificity.

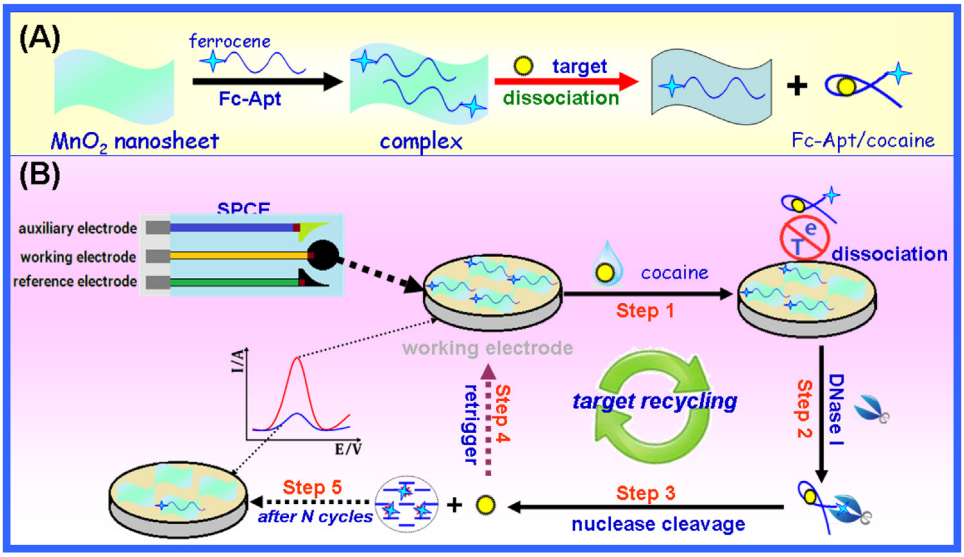
2. Experimental

2.1. Material and reagent

DNase I from bovine pancreas (Type IV, lyophilized powder, ≥ 2000 Kunitz units mg^{-1} protein) and cocaine hydrochloride was purchased from Sigma-Aldrich (USA). All these oligonucleotides including ferrocene-labeled cocaine aptamer (5′-ferrocene-GACAAGGAAAATCCTTCAATGAAG TGGGTC-3′, designated as Fc-Apt) were ordered from Sangon Biotechnol. Co., Ltd. (Shanghai, China) (*Note*: The sequence was selected referring to the literature [20]). Cetyltrimethylammonium bromide (CTAB) was achieved from Genview (USA). All other reagents including potassium permanganate ($KMnO_4$) were of analytical grade. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ cm^{-1}, Milli-Q, Millipore) was used in all runs. Phosphate buffer was prepared by using 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and 0.1 M KCl was used as the supporting electrolyte. DNA stocking solution was initially heated to 90 °C for 5 min and then cooled to room temperature prior to use (*Note*: The aim of using this step is to ensure all the oligonucleotides with the single-stranded DNA). All the screen-printed carbon electrodes (SPCE) consisting of a carbon working electrode (effective area: 6.28 mm^2), an Ag/AgCl reference electrode and a graphite auxiliary electrode were acquired from Metrohm Inc. (Switzerland).

2.2. Preparation of MnO_2 nanosheets

Prior to modification, MnO_2 nanosheets were initially prepared *via* a typical hot-injection method [21]. Briefly, 10 mL of ultrapure water including 0.3644 g CTAB was initially heated to 140 °C in the oil bath under vigorous stirring, and then 10 mL of $KMnO_4$ aqueous solution including 31.6 mg $KMnO_4$ was quickly added to the boiling solution, which was reacted for 120 min at above-mentioned temperature. Afterwards, the resulting suspension was centrifuged for 10 min at 12,000*g* and washed with acetone. After repeating (centrifugation and washing) for three times, MnO_2 nanosheets were obtained for further use.



Scheme 1. Schematic representation of target-responsive aptamer dissociation from MnO_2 nanosheets-modified screen-printed carbon electrode (SPCE) for electrochemical sensing of cocaine with target recycling amplification: (A) target-induced aptamer dissociation and (B) target recycling process.

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