Analytica Chimica Acta 719 (2012) 76–81

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

Aptamer sensor for cocaine using minor groove binder based energy transfer

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ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form
30 December 2011
Accepted 3 January 2012
Available online 13 January 2012

Keywords:
Aptamer
Minor groove binder based energy transfer
Cocaine
Polydimethylsiloxane

ABSTRACT

We report on an optical aptamer sensor for cocaine detection. The cocaine sensitive fluorescein isothiocyanate (FITC)-labeled aptamer underwent a conformational change from a partial single-stranded DNA with a short hairpin to a double-stranded T-junction in the presence of the target. The DNA minor groove binder Hoechst 33342 selectively bound to the double-stranded T-junction, bringing the dye within the Förster radius of FITC, and therefore initiating minor groove binder based energy transfer (MBET), and reporting on the presence of cocaine. The sensor showed a detection limit of 0.2 μM. The sensor was also implemented on a carboxy-functionalized polydimethylsiloxane (PDMS) surface by covalently immobilizing DNA aptamers. The ability of surface-bound cocaine detection is crucial for the development of microfluidic sensors.

1. Introduction

Cocaine, or benzoylmethylecgonine, is a common illicit drug encountered by law enforcement, border protection and forensic science authorities [1,2]. Current field tests for cocaine are either presumptive and require confirmatory analysis in the laboratory or necessitate high levels of training for effective operation [3]. Chemical sensors for cocaine which are simple to operate and still offer high sensitivity and specificity are therefore required. A range of chemical sensors has been developed in recent years involving fluorescence [4–12], colorimetric [13–21], chemiluminescence [22–24], electrochemical [17,25–37], surface-enhanced Raman scattering [38–40], surface plasmon resonance [31,41] and surface acoustic wave [42,43] based transducers. Fluorescence- and colorimetric-based sensors are particularly desirable due to the simple detection procedures involved [16].

Stojanovic et al. [4] were the first to report the use of aptamers for the detection of cocaine in fluorescence-based sensors. Aptamers are nucleic acid based receptors that are obtained through a combinatorial selection process known as systematic evolution of ligands by exponential enrichment (SELEX) [44,45]. These molecules have significant advantages over antibodies, such as convenient synthesis and chemical modification, high affinity even to small molecular targets and resistance to biodegradation [14,22,26,46]. Among fluorescence-based aptamer sensors, fluorescence resonance energy transfer (FRET)-based aptamer sensors are particularly attractive for cocaine detection because of the inherent sensitivity of FRET to detect conformation-associated change in donor/acceptor dye separation [8]. In the presence of cocaine, FRET between fluorescein and dabcyl dye both attached to the aptamer was stimulated by the formation of a ligand-induced binding pocket based on terminal stem-closure [4,5]. Quantum dot (QD)-stimulated FRET with organic dyes on DNA aptamers was recently reported for cocaine detection [7,47]. Furthermore, Cy5 labeled cocaine aptamers were hybridized with complementary DNA attached to gold nanoparticles. In the presence of cocaine, the aptamer strands were released resulting in deactivation of FRET between the gold nanoparticles and Cy5, and recovery of Cy5 fluorescence [8].

It is generally acknowledged that microfluidic devices are particularly useful for the implementation of lab-on-a-chip sensors due to reduced sample and reagent consumption, shorter analysis times and increased levels of automation [48]. Hilton et al. [11] first used a polydimethylsiloxane (PDMS)/glass based microchamber packed with aptamer-functionalized microbeads as a FRET-based sensor for cocaine detection.

Here, we demonstrate an optical cocaine sensor where a cocaine-sensitive aptamer labeled with FITC changes conformation from a partial single-stranded oligonucleotide with a short hairpin to a double-stranded T-junction, thereby trapping cocaine. The double-strand specific DNA minor groove binder Hoechst 33342 binds to the double-stranded T-junction allowing the dye upon excitation at 360 nm to instigate energy transfer to FITC. The result of this effect which we term minor groove binder based energy transfer (MBET) is green fluorescence at 520 nm.

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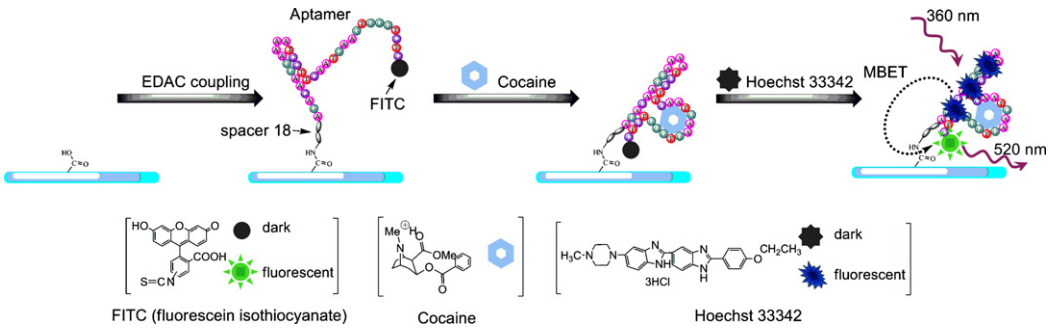
doi:10.1016/j.aca.2012.01.011



Scheme 1. Schematic illustration of MBET aptamer sensors for cocaine detection on UDA modified PDMS surface.

We also demonstrate the MBET cocaine sensor on a PDMS surface for ready integration into microfluidic chips. We fabricate a carboxy-functionalized PDMS surface by simply curing a mixture of undecylenic acid (UDA) and PDMS prepolymer on a gold coated glass slide, which had been pretreated with a hydrophilic self-assembled monolayer (SAM) of 3-mercaptopropionic acid (Scheme S-1). FITC labeled 5′-amino-terminal single-stranded DNA aptamers are covalently attached to the UDA modified PDMS surface *via* amide linkages (Scheme 1).

2. Experimental

2.1. Materials and reagents

PDMS Sylgard 184 was purchased from Dow Corning Corporation (USA) as a two-component kit, including pre-polymer (base agent) and cross-linker (curing agent). Gold slides were purchased from Platypus Technologies (USA). Lucifer Yellow CH dipotassium salt, Hoechst 33342 were purchased from Invitrogen (USA). Cocaine was obtained from Forensic Science, Adelaide, South Australia, Australia. All other chemicals were purchased from Sigma–Aldrich (USA). The aptamer sequence used here was based on a literature sequence [5,7] and was adapted to suit our application. 5′-amino-(spacer18)$_2$ AGACAAGGAAAATCCTTCAATGAAGTGGGTCTC-FITC-3′ was purchased from GeneWorks Pty Ltd (Australia). The buffers were prepared as follows: PBS (pH 4.8): NaH_2PO4 (30 mM), 10 mM PBS (pH 7.4), NaCl (137 mM), KCl (54 mM), Na_2HPO4 (10 mM), KH_2PO4 (2 mM) and tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.4): Tris (25 mM), NaCl (100 mM), $MgCl_2$ (1 mM). For pH adjustment of the buffers, NaOH (0.1 M) and HCl (0.1 M) were used.

2.2. PDMS sample preparation

The UDA modified PDMS surface was prepared according to our previous work [49]. Briefly, the PDMS (10:1 weight ratio of base and curing agents) and 2 wt% UDA were thoroughly mixed and degassed to remove air bubbles and then poured onto the hydrophilic gold slide which was pre-coated with a 3-mercaptopropionic acid monolayer. The sample was left on the bench under ambient condition for 24 h and then cured at 80 °C for 2 h. Following this, the sample was immersed in MilliQ water for 4 h so that the cured UDA modified PDMS can be easily peeled off from the gold substrate. The UDA modified PDMS was finally rinsed sequentially with MilliQ water and ethanol and dried under a stream of nitrogen (Scheme S-1). Native PDMS without UDA was used as a control.

2.3. Surface characterization

2.3.1. X-ray photoelectron spectroscopy (XPS)

XPS analysis of PDMS samples was performed on an AXIS HSi spectrometer (Kratos Analytical Ltd, GB), equipped with a monochromatized Al Kα source. The pressure during analysis was typically maintained at 5×10^{-9} kPa. High resolution spectra were collected at a pass energy of 40 eV. Binding energies were calibrated against the aliphatic hydrocarbon peak at 285.0 eV.

2.3.2. Streaming zeta-potential analysis

Zeta potential data were obtained using a ZetaCAD instrument equipped with an RS232 C bi-directional interface as well as a programmable in/out board for automation of the measurements with the aid of a Keithley 2400 high accuracy multimeter. The approach published by Karkhaneh et al. [50] was adopted where potassium chloride (1 mM) was used as a background electrolyte in all experiments. Potassium hydroxide (0.1 M) and hydrochloric acid (0.1 M) were used for pH adjustment. The tested PDMS substrates were immersed in the electrolyte solution overnight for equilibration prior to testing. The measurements were repeated three times at pH 4 to pH 12 at room temperature, and the results were averaged.

2.3.3. Fluorescence labeling

The presence of carboxylic acid groups on the UDA modified PDMS surface was confirmed by fluorescence labeling using the Lucifer Yellow CH dipotassium salt dye. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and Lucifer Yellow CH dipotassium salt dye were dissolved in MilliQ water (0.4 M EDAC and 1 mg mL^{-1} dye). Native PDMS and UDA modified PDMS samples were immersed into the EDAC/dye solution for 4 h at room temperature after which the samples were removed and rinsed sequentially with MilliQ water and ethanol and finally dried under a stream of nitrogen gas. The samples were then imaged under a fluorescence microscope (Leitz Laborlux fluorescence microscope).

2.3.4. Other analyses

In addition, water contact angle (WCA) measurements and Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy analysis were also performed on native PDMS and the UDA modified PDMS surfaces [49].

2.4. MBET aptamer sensor for cocaine detection in solution

To optimize the temperature protocol for cocaine detection using the FITC-labeled cocaine aptamer, three parallel experiments were performed. (a) A solution containing aptamer (0.1 μM) and cocaine (0.1 μM) in Tris buffer (pH 8.4) was maintained at room temperature for 20 min; (b) A solution containing