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A novel electrochemical method to detect theophylline utilizing silver ions captured within abasic site-incorporated duplex DNA

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ABSTRACT

We herein describe a novel and label-free electrochemical system to detect theophylline. The system was constructed by immobilizing duplex DNA containing an abasic site opposite cytosine on the gold electrode surface. In the absence of theophylline in a sample, silver ions freely bind to the empty abasic site in the duplex DNA leading to the highly elevated electrochemical signal by the redox reaction of silver ions. On the other hand, when theophylline is present, it binds to the abasic site by pseudo base pairing with the opposite cytosine nucleobase, which consequently prevents silver ions from binding to the abasic site. As a result, redox reaction of silver ions would be greatly reduced resulting in the accordingly decreased electrochemical signal. By employing this electrochemical strategy, theophylline was reliably detected at a concentration as low as 3.2 μM with the high selectivity over structurally similar substances such as caffeine and theobromine. Finally, the diagnostic capability of this method was also successfully verified by reliably detecting theophylline present in a real human serum sample with an excellent recovery ratio within 100 ± 6%.

1. Introduction

Theophylline is one of the most commonly used bronchodilators and respiratory stimulators for the treatment of acute and chronic obstructive pulmonary disease (Barnes, 2003; Kawai and Kato, 2000). The therapeutic window for theophylline in serum is narrow with an effective concentration range of 60–100 μM (Hendeles and Weinberger, 1983). Higher concentration of theophylline over the range could cause lethal or neurological damages (Ferapontova and Gothelf, 2009b). Therefore, accurate and careful monitoring of the theophylline concentration in serum is highly demanded to prevent its toxic side effect. Currently, detection of serum theophylline has been carried out in many clinical laboratories by employing routine methods such as gas or liquid chromatography, ultraviolet spectrometry, and immunoassay (Mounie et al., 1990). However, these methods suffer from several limitations including the need for technical expertize as well as the long analysis time required for complex and multi-step procedures. In addition, selectivity of these methods is relatively low over structurally similar molecules such as caffeine or theobromine, which could cause overestimated results.

In order to overcome these limitations, an RNA aptamer-based sensing system has emerged as an alternative approach for the detection of theophylline (Jenison et al., 1994). Taking advantages of RNA aptamer exhibiting high binding affinity and selectivity toward theophylline, various signaling strategies have been developed by employing colorimetric (Pernites et al., 2011), fluorescent (Rankin et al., 2006; Stojanovic and Kolpashchikov, 2004), and electrochemical (Ferapontova and Gothelf, 2009a; Ferapontova et al., 2008) signaling methods. However, RNA molecules are basically not considered as a good sensing component because RNA synthesis is more difficult and expensive than DNA and also susceptible to the cleavage by ubiquitous ribonuclease and chemical reagents, which consequently impedes the widespread use of RNA aptamer-based assay.

Recently, Teramae et al. discovered a new class of DNA duplex aptamer to recognize theophylline where abasic (AP) site is incorporated serving as an active and strong binding receptor for theophylline (Nishizawa et al., 2010; Sankaran et al., 2006; Sato et al., 2009, 2012). This DNA duplex aptamer shows high binding affinity toward theophylline only when cytosine is positioned opposite AP site and discriminates theophylline very selectively from structurally similar substances such as caffeine and theobromine (Sankaran et al., 2006). Utilizing the unique feature, they developed a new strategy to detect theophylline, which relies on the DNA duplex probe modified with fluorescent analog of adenine, 2-aminopurine, near the AP site. Theophylline bound into the AP site changes the microenvironment of fluorescent 2-aminopurine, which consequently results in the significant reduction of fluorescence signal from 2-aminopurin (Pang et al.,

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2012). Although interesting, the need for the modification of DNA probe with 2-aminupurine might make the assay a little complicated and expensive (Li et al., 2009).

In an alternative approach aimed at eliminating the above limitations, Park et al. (2014) recently developed a modification-free method to detect theophylline by utilizing fluorescent silver nanocluster (AgNCs) as a signaling unit. In this method, the formation of AgNCs within AP site opposite cytosine moiety is controlled by competitive binding of theophylline. The strategy is quite convenient yielding high sensitivity and selectivity toward target theophylline. However, the fluorescent signal of the AgNCs might be significantly reduced by several substances present in a blood serum sample, which inevitably hinders the widespread use of the method by essentially requiring the pretreatment step to remove undesirable substances from blood serum. Therefore, there is still a significant challenge for development of novel strategy to detect theophylline in a highly stable, efficient, and cost effective manner.

In order to meet these demands, we designed a new electrochemical method to detect theophylline that could be much cheaper and more convenient than the previously reported fluorescence-based methods (Park et al., 2014; Rankin et al., 2006; Stojanovic and Kolpashchikov, 2004), which enables more widespread application even in facility-limited environments (Burke and Gorodetsky, 2012; Won et al., 2011). In this strategy, the surface of electrode is modified with the DNA duplex aptamer possessing AP-site opposite cytosine nucleobase. The electrochemical sensor is operated by generating a signal through the redox reaction of silver ions captured into the AP site (Shao et al., 2009), which is regulated by the competitive binding of theophylline into the identical AP site. This is the first report to detect theophylline in a very convenient electrochemical manner overcoming the limitations associated with the previous methods.

2. Materials and methods

2.1. Materials

All synthetic oligonucleotides were synthesized and purified by high-performance liquid chromatography (HPLC) from Integrated DNA Technologies (IDT, Coralville, USA) (Table S1). The AP site-incorporated DNAs were modified with thiolate (–SH) functional group at 5′ end. Silver nitrate, theophylline, caffeine, theobromine, creatinine, D-glucose, human serum, sodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and used without further purification (Park et al., 2013). Aqueous solutions were prepared using ultrapure DNase/RNase-free distilled water (Carlsbad, CA, USA).

2.2. Preparation of gold electrode platform

The coating of titanium (20 nm) on Si wafer (100) was followed by 200 nm gold (99.999%) thin layer formation by an e-beam evaporator. The gold-coated electrode surface was immersed into piranha solution (H_2SO_4:H_2O_2=4:1) for 15 min (Baek et al., 2013). The electrode surface was then thoroughly washed with PBS (50 mM sodium phosphate, 75 mM sodium chloride, pH 7.2) and dipped into the 200 μL of aqueous solution containing 1 μM AP site-incorporated DNA (Table S1) for 4 h. After washing the electrode with PBS, 1 mM mercaptohexanol solution was applied onto the electrode surface for 30 min to block bare surfaces uncovered by the AP site-incorporated DNA. Finally, the modified electrode with a surface coverage of 12.3×10^{12} molecules/cm^2 were washed with PBS and water, and dried under N_2 atmosphere. The amount of DNA immobilized on the electrode was determined to be 12.3×10^{12} molecules/cm^2 based on the Beer Lambert Law by measuring the absorbance difference of the DNA solution at 260 nm before and after immobilization process.

2.3. Theophylline detection procedure

200 μL of 1 μM complementary single-stranded DNA having different bases opposite the AP site (Table S1) was allowed to hybridize with the AP site-incorporated DNA (AP-middle) on the gold electrode surface for 1 h at 60 °C. The solution was then cooled down to 25 °C and the electrode was washed with PBS. A solution containing theophylline at different concentrations or theobromine, caffeine, D-glucose, and creatinine at 250 μM was applied to the gold electrode and incubated for 45 min at 15 °C. After washing the electrode surface with PBS and water, the electrode was immersed into the solution containing 10 μM $AgNO_3$, incubated for 45 min and again completely washed with PBS and water. Detection procedure described above is summarized in Fig. S1 for a better understanding.

2.4. Electrochemical measurement

The gold electrode was immersed into 1 mL of phosphate buffer (20 mM) containing 100 mM sodium acetate for 10 min before electrochemical measurement. The gold matrix electrode was used as a working electrode with Ag/AgCl reference electrode and platinum counter electrode. Cyclic voltammetry (CV) was performed using a reference 600 electrochemical analyzer (GAMRY, Warminster, PA, USA) connected to a computer for data analysis from 0.5 V to −0.5 V using 10–200 mV s^{-1} scan rates, and the CV under 100 mV s^{-1} was used to detect and quantify target theophylline.

2.5. Preparation and analysis of Human serum samples

A human serum sample diluted 20 times with the phosphate buffer (20 mM phosphate, 100 mM sodium acetate, pH 7.4) containing various concentrations of theophylline was directly applied to the electrode. The sample was analyzed by following the above-mentioned detection procedure. To determine the amount of theophylline, the calibration curve was first constructed by a set of standards containing a known amount of theophylline in human serum. The electrochemical peak current from the unknown samples was measured and interpolated to determine the concentration of theophylline present in human serum based on the calibration curve.

3. Results and discussion

3.1. The overall detection procedure

The overall procedure for electrochemical system to detect theophylline is illustrated in Fig. 1. The AP site within duplex DNA immobilized on the surface of electrode serves as a binding pocket for theophylline through pseudo base pairing with the opposite cytosine that is stabilized by nucleobases adjacent to the AP site (Li et al., 2009; Pang et al., 2012). At the same time, the cytosine nucleobase positioned opposite the AP site also captures silver ions through its strong interaction (Ma et al., 2011; Park and Park, 2014) when the AP site is not occupied by target theophylline (Fig. 1). The silver ion captured within the AP site then produces electrochemical signal through its redox reaction on the electrode surface. On the other hand, when theophylline is paired with cytosine base in the AP site, it prevents silver ions from binding to