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A highly sensitive gold nanoparticle-based electrochemical aptasensor for theophylline detection

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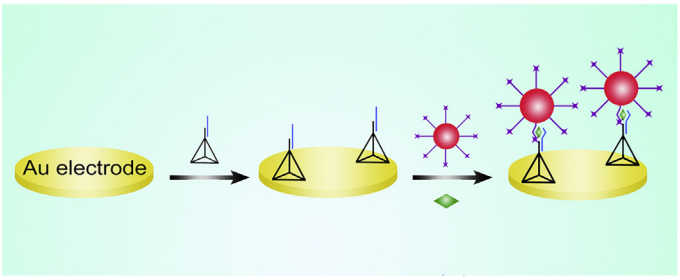
HIGHLIGHTS

- An electrochemical aptasensor for theophylline analysis is developed.
- DNA tetrahedron is modified on the electrode sensing interface.
- Gold nanoparticles-assisted amplification is involved.
- The developed aptasensor is highly selective for the detection of theophylline.

GRAPHICAL ABSTRACT



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ABSTRACT

Theophylline is a common bronchodilator for the treatment of diseases like asthma, bronchitis and emphysema. However, it should be strictly used and monitored due to its toxicity when the concentration is above certain levels. In this work, an electrochemical biosensor for theophylline detection is proposed by recognition of RNA aptamer and gold nanoparticle (AuNP)-based amplification technique. First, RNA aptamer is splitted into two single-stranded RNA probes. One is hybridized with DNA tetrahedron and the resulted nanostructure is then immobilized onto a gold electrode; the other is modified on the surface of AuNPs which is also labeled with methylene blue (MB) as electrochemical species. The recognition process between the two RNA probes and theophylline causes the localization of AuNPs and the enrichment of MB on the electrode interface. A significant electrochemical response is thus generated which is related to the concentration of initial theophylline. This proposed aptasensor shows excellent sensitivity and selectivity which could also be applied in quantitatively detection of theophylline in serums samples.

1. Introduction

Theophylline is a popular drug for the treatment of many diseases such as chronic obstructive pulmonary disease (COPD), neonatal apnea, and asthma, which has been clinically used for more than 80 years [1]. Abundant evidences have indicated that low-dose theophylline possesses anti-inflammatory and immunomodulatory effects in certain diseases like COPD [2,3]. Moreover,

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the cost of theophylline is low, thus it is widely used in developing countries. However, some adverse effects of theophylline exist when the serum concentration is above 20 μg/mL [4]. A narrow therapeutic range is from 5 to 15 μg/mL [5]. Therefore, there is a considerable interest in biological analysis. Although a number of analytical methods have been developed for the determination of theophylline [6–8], unfortunately, some disadvantages do exist including poor time resolution, poor limit of detection, complicated operation and poor detection stability. Furthermore, large sample volumes, complex solvents, expensive instruments may be required in most cases.

Electrochemical techniques have the merits of high sensitivity, low cost and rapid process, which are ideally suited for theophylline assays [9–11]. For instance, Ferapontova et al. developed an electrochemical aptasensor for theophylline using methylene blue (MB) as electrochemical species [12]. RNA aptamer was immobilize on the electrode surface, the conformation of which was changed upon theophylline binding. Accordingly, MB is getting closer to the electrode surface and the electrochemical response was increased to probe the level of target theophylline. However, this electrochemical sensor was preliminary without any signal amplification elements, which could only respond theophylline with a relative high concentration. Kumar et al. constructed a non-enzymatic electrochemical method for the determination of theophylline [13]. Voltammetric behavior was recoded on a para amino benzene sulfonic acid modified glassy carbon electrode, which standed for the oxidation of theophylline. Nevertheless, the detection can be easily interfered by other electroactive species and the selectivity may be limited. Therefore, there is still urgent need to develop more advanced electrochemical methods for theophylline analysis.

Nanomaterials sized between 1 and 100 nm have unique physical or chemical properties [14–16]. They have been applied in a number of analytical methods with excellent electrochemical behaviors such as silver nanoparticles and copper nanoparticles [17,18]. In this contribution, gold nanoparticles (AuNPs) with high specific surface area and excellent electrical conductivity are utilized for amplified electrochemical sensing of theophylline [19]. Briefly, DNA tetrahedron nanostructure has been immobilized on the electrode surface in order to improve molecular recognition efficiency and avoid redundant electrode modification steps [20]. Since split aptamers may help reduce the background and guarantee the high selectivity [21–24], the recognition event occurs herein is conducted between theophylline and two split RNA aptamers on DNA tetrahedron and AuNPs respectively, instead of using a single-stranded RNA aptamer [12]. In addition, AuNPs process huge specific surface area for RNA probe immobilization. As a result, the attached MB molecules on the RNA probe could be enriched to a great extent. Moreover, AuNPs have excellent electrical conductivity, thus, significant electrochemical signal can be obtained [25]. The developed electrochemical method for theophylline shows high sensitivity with the AuNP-based signal amplification. The linear range is obtained from 0.1 to 80 μM with the limit of detection (LOD) as low as 0.07 μM.

2. Experimental

2.1. Materials and chemicals

Chloroauric acid ($HAuCl_4$) and trisodium citrate were purchased from Shanghai Jiushan Chemicals Co., Ltd. (Shanghai, China). Ethylenediaminetetraacetic acid (EDTA), tris(2-carboxyethyl)phosphinehydrochloride (TCEP) and diethypyrocarbonate (DEPC) were obtained from Sigma (USA). 20 bp DNA Ladder, DNA probes and RNA probes were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China), the sequences of which were listed in Table 1. Other reagents were of analytical grade and were used as received. Double-distilled water used to prepare all solutions was purified with a Millipore system under 18 MΩ cm resistivity and then treated with DEPC.

2.2. Preparation of RNA probe b modified AuNPs

AuNPs were synthesized by the citrate reduction of $HAuCl_4$ according to a previous report [26]. A typical process was as follows. 3.5 mL of 1% (w/v) trisodium citrate was freshly prepared and added to 100 mL of 0.01% (w/v) $HAuCl_4$ under violent stirring. The solution was boiling for 15 min and the stirring was stopped after another 30 min. Subsequently, the prepared AuNPs were cooled down to room temperature, which were then purified by three cycles of centrifugation at 12, 000 rpm for 30 min. The functionalization of AuNPs with RNA probe b was achieved by gold–sulfur chemistry [27]. Briefly, 4 μM RNA probe b was added to 1 mL of AuNPs with the concentration of 10 nM. After 16 h, the RNA-AuNPs conjugates were "aged" in salts (0.1 M NaCl, 10 mM phosphate, pH 7.0) for 24 h, which made the RNA molecules on the surface of AuNPs vertical and ordered. Next, the RNA modified AuNPs were purified by centrifuging at 12, 000 rpm for 30 min.

2.3. Gold electrode treatment and modification

The substrate gold electrode (3 mm diameter) was soaked in piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1) for 5 min (*Caution: Piranha solution was highly corrosive and reacts violently with organic matter!*). After rinsed with double-distilled water, the electrode was carefully polished to a mirror-like surface on P3000 silicon carbide paper and then 1, 0.3 0.05 μm alumina slurry, respectively. Subsequently, it was sonicated for 5 min in both ethanol and double-distilled water in order to remove the residual alumina powder. The electrode was soaked in nitric acid (50%) for 30 min and then electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities. The pretreated electrode was ready for further modification.

Tetrahedral nanostructure was formed through partially hybridization events between four single-stranded DNA probes (DNA probe a, b, c, d) and RNA probe a. Briefly, each strands with the concentration of 5 μM was prepared in the 10 mM Tris-HCl buffer solution (pH 7.0) containing 10 mM TCEP and 50 mM $MgCl_2$, respectively. The five solutions were heated to 95 °C for 2 min to

Table 1
Sequences of nucleic acids used in this work.

Name	Sequence (from 5′ to 3′)
DNA probe a	CCAGCCGAAAACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
DNA probe b	SH-C_6-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
DNA probe c	SH-C_6-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
DNA probe d	SH-C_6-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
RNA probe a	CGGCUGGGGCGAUACCAGCCGAAA
RNA probe b	SH-C_6-CCAGCCGAAAGGCCCUUGGCAGCGUCGGG-MB

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