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Aptamer-based biosensor for label-free detection of ethanolamine by electrochemical impedance spectroscopy

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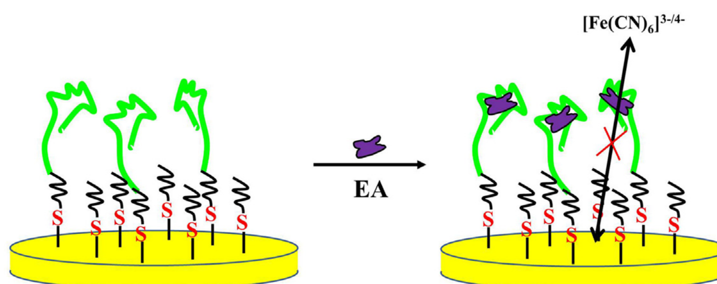
HIGHLIGHTS

- Label-free aptamer DNA sensor is developed for EA detection.
- Aptamer switches to G-quadruplex on the electrode, offering the binding site of EA.
- CD and EIS confirm the sensing mechanism.
- The sensor exhibits excellent selectivity and high sensitivity.
- The sensor is successfully challenged in serum and tap water samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A label-free sensing assay for ethanolamine (EA) detection based on G-quadruplex-EA binding interaction is presented by using G-rich aptamer DNA (Ap-DNA) and electrochemical impedance spectroscopy (EIS). The presence of K^+ induces the Ap-DNA to form a K^+-stabilized G-quadruplex structure which provides binding sites for EA. The sensing mechanism was further confirmed by circular dichroism (CD) spectroscopy and EIS measurement. As a result, the charge transfer resistance (R_{CT}) is strongly increased as demonstrated by using the ferro/ferricyanide ($[Fe(CN)_6]^{3-/4-}$) as a redox probe. Under the optimized conditions, a linear relationship between ΔR_{CT} and EA concentration was obtained over the range of 0.16 nM and 16 nM EA, with a detection limit of 0.08 nM. Interference by other selected chemicals with similar structure was negligible. Analytical results of EA spiked into tap water and serum by the sensor suggested the assay could be successfully applied to real sample analysis. With the advantages of high sensitivity, selectivity and simple sensor construction, this method is potentially suitable for the on-site monitoring of EA contamination.

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1. Introduction

Organic amines are a class of ubiquitous environmental contaminants with high mutagenicity, teratogenicity and carcinogenicity [1,2]. The carcinogenesis of the amines is mainly due to the highly reactive and electrophilic intermediates produced by N-

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hydroxylation of the amine group in metabolic activation process [3,4], as the produced intermediates can interact with nucleobases to form DNA adducts and produce different types of lesions [5,6], which can induce mutation in the process of DNA replication and transcription, and finally initiating cancer disease [7]. Ethanolamine (EA), one of the smallest molecules of aliphatic amine with simple structure, plays very important roles in textile, chemical industries, pharmaceuticals, biology science, and has been widely used in the production of dyes and pesticides intermediates, insecticide, medicine, adhesives, rubber-manufacturing processes (rubber accelerator, vulcanizing agent of the rubber) and surfactant. However, studies showed that human exposure to EA can irritate the skin, eyes and cause damage to the respiratory tract [8]. Furthermore, EA can also inhibit the nervous system when it entered the human body. Besides, it's confirmed that EA is associated with Alzheimer's disease [9], Schizophrenia [10], and the carcinogenic activity of EA is investigated as well [11]. So it is necessary to develop efficient methods for the determination of EA.

Commonly, high-performance liquid chromatography (HPLC) with UV and/or fluorescence detection method is the technique that widely used for detection of small molecules [11]. However, these methods are relatively complicated; require expensive equipments, time-consuming pre-treatment of the sample prior to analysis, highly qualified technicians and a laboratory environment. Under this situation, an achievement of simple and cost-effective assay is imperative [12]. Electrochemical technique is an interesting and convenient alternative that has allowed one to simplify the detection process [13], and is ease to miniaturize resulting in low consumption of nucleic acid aptamers [14]. Especially, the interaction between DNA and the target molecules provides the basis for analytical devices in laboratory [15]. So electrochemical DNA sensors possess enormous potential for the task of rapid, simple and sensitive detection of target molecules [16,17] due to their convenience, high selectivity and sensitivity. And electrochemical impedance spectroscopy (EIS) has been proven to be a most powerful and sensitive tool for probing the features of surface-modified electrodes [18]. EIS biosensors possess unique advantages, such as the ability to separate the surface binding events from the solution impedance, ease of signal quantification, less damage to the biological interactions being measured, and the possibility to use non-labeled DNA [19].

Up to date, little efforts have been given to develop DNA sensors for amine compounds, and only a few DNA sensor for the detection of the aromatic amines based on the binding interaction have been reported [20,21]. For example, Liang et al. reported a hairpin DNA modified sensor for the electrochemical determination of amino-substituted naphthalene compounds with high sensitivity, and further confirmed the binding mode of the aminonaphthalenes with hairpin DNA [12]. However, all these electrochemical DNA sensors showed poor selectivity to the target amine compounds, and can not discriminate between different amine compounds. So developing sensors for amine compounds with high selectivity is still of a great challenge.

In contrast, aptamer exhibits high affinity to specific targets. Thus, aptamers become important components in the design of biosensors, and aptamer-based sensors have been widely used for targeting small molecule [22], such as cocaine [23], organophosphorus pesticides [24,25], heavy metals [26], pharmaceuticals [27,28], due to the considerable advantages, such as thermal stability, simpler preparation, lower-priced production cost, high selectivity and sensitivity for targets [22,29]. Until now, EA aptamer, one of the smallest aptamer targets so far, has been in vitro-selected [30], but few aptamer sensors have been constructed for EA detection. On the basis of the findings, Heilkenbrinker group reported an aptamer-based microarray assay based on competition between EA and the fluorescein-labeled DNA strand, which is complementary to the aptamer DNA sequence immobilized on the microarray surface [11]. This methods made great improvements in detection techniques for EA. However, the signal-off fluorescent sensor has a major drawback in that the fluorescence signal can be easily affected by matrixes existed in the testing system, which limit the scope of their applications in environmental and biological samples. Therefore, it still remains a challenge to achieve simple, cost-effective, label-free determination of EA with high capacity of resisting disturbance, which could really meet the requirements for EA detection in real samples. So developing aptamer DNA-based sensors with high specificity, sensitivity is of great significance.

In the present work, a label-free sensing assay for the detection of EA was reported based on aptamer DNA (Ap-DNA) sensor by electrochemical impedance spectroscopy (EIS). Simply, a hairpin Ap-DNA was first immobilized on the gold electrodes and transformed into G-quadruplex, which can bind with EA. The interaction mechanism was further confirmed by circular dichroism (CD) spectroscopy and EIS. Depending on the difference of charge transfer resistance change (ΔR_{CT}) before and after reaction with EA, EA can be sensitively and selectively detected with a detection limit of 0.08 nM in buffer solution. Finally, the Ap-DNA assay was successfully performed in natural tap water and serum samples.

2. Materials and methods

2.1. Materials

The following DNA sequences (the thiolated arbitrary single-stranded DNA and aptamer DNA of EA) were purchased from Shanghai Sangon Biological Engineering Technology & Service Co. Ltd (http://www.sangon.com).

5′-HS-$(CH_2)_6$-TTTTTTTATTCAAGAGGTGGGT-3′ (ss-DNA) 5′-HS-$(CH_2)_6$-TTTTTTATTCAA**TTTGAGGCGGGTGGGTGGG**TTGAATA-3′ (Ap-DNA) 6-Mercapto-1-hexanol (MCH), $NaClO_4$, Tris (tris-(hydroxymethyl)-aminomethane) (Tris), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, EDTA, KCl, $MgCl_2$, $[Ru(NH_3)_6]Cl_2$, $[Ru(NH_3)_6]Cl_3$ were purchased from Sigma-Aldrich (http://www.sigmaaldrich.com/china-mainland.html) and used without further purification. Ethanolamine (EA), ethanol, glycol, isopropanol, isopropanolamine (IPA), HCl, $HClO_4$, NaCl, $CaCl_2$ were purchased from Sinopharm Chemical Reagents Beijing Co., Ltd (http://www.crc-bj.com). Fetal calf serum (FBS) was obtained from JIBCO (Cat: 10099-141, Invitrogen, MA, USA). The working gold electrodes, 99.99% (w/w) polycrystalline with a diameter 1 mm, were obtained from Aida Instrument Inc. in Tianjin (China) (http://www.tjaida.cn) and cleaned prior to use as reported before.

The stock solutions of thiolated DNA (10 μM) was prepared in Tris-HCl buffer (20 mM, pH = 7.4) and then heated at 80 °C for 5 min to remove possible DNA aggregates and to dissociate any intermolecular interaction, obtaining uniform single-stranded DNA solution and gradually cooled to room temperature prior to use [19]. And then, the DNA stock solution was diluted to a final concentration of 1 μM with the DNA immobilization buffer (10 mM Tris-HCl, 1.0 M NaCl, 1 mM EDTA, 1 mM TCEP, pH 8.0). TCEP was used to cleave disulfides. Besides, the Tris-HCl buffer (20 mM, pH = 7.6) containing 100 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 0.02% Tween was prepared and used as binding buffer (B-buffer) for Ap-DNA and EA. EA was diluted with B-buffer to obtain a series of EA solutions with different concentration prior to use. Serum sample was prepared by diluting with B-buffer with a final concentration of 10% serum, and then for EA solution preparation. Tap water was first purified with the filter membrane (0.45 μm),

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