Analytica Chimica Acta xxx (2018) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Electrochemical screening of single nucleotide polymorphisms with significantly enhanced discrimination factor by an amplified ratiometric sensor

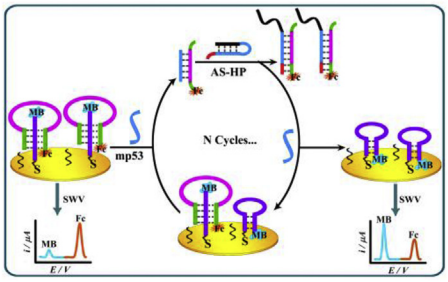
Baoting Dou [a], Jin Li [b], Bingying Jiang [b, **], Ruo Yuan [a], Yun Xiang [a, *]

[a] Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] School of Chemistry and Chemical Engineering, Chongqing University of Technology, Chongqing 400054, PR China

HIGHLIGHTS

- Sensitive electrochemical detection of SNP is realized by a ratiometric sensor.
- The ratiometric signal is significantly amplified by enzyme-free target recycling.
- The developed sensor shows substantially improved SNP discrimination factor.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 December 2017
Received in revised form
14 June 2018
Accepted 10 July 2018
Available online xxx

Keywords:
Ratiometric sensor
Single nucleotide polymorphisms
Signal amplification
Triple helix complex
Enhanced discrimination factor

ABSTRACT

The detection of single nucleotide polymorphisms (SNPs) is of great clinical significance to the diagnosis of various genetic diseases and cancers. In this work, the development of an ultrasensitive ratiometric electrochemical sensor for screening SNP with a significantly enhanced discrimination factor is reported. The ferrocene (Fc) and methylene blue (MB) dual-tagged triple helix complex (THC) probes are self-assembled on the gold electrode to construct the sensing interface. The addition of the mutant p53 gene causes the disassembly of the THC probes with the release of the Fc-tagged sequence and the folding of the MB-labeled sequence into a hairpin structure, causing the change in the current response ratio of MB to Fc for monitoring the mutant p53 gene. Such ratio is dramatically enhanced by the toehold-mediated displacement reaction-assisted target recycling amplification with the presence of an assistance hairpin sequence. With the significant signal amplification and the advantageous specificity of the THC probes, sub-femtomolar detection limit and a highly enhanced SNP discrimination factor for the mutant p53 gene can be obtained. Besides, the proof-of-demonstration application of the sensor for diluted real samples has been verified, offering such sensor new opportunities for monitoring various genetic related diseases.

1. Introduction

The ratiometric methodology relies on measuring changes of the ratio of two independent signals for detecting the targets of

* Corresponding author.
** Corresponding author.
E-mail addresses: jiangby@cqut.edu.cn (B. Jiang), yunatswu@swu.edu.cn (Y. Xiang).

https://doi.org/10.1016/j.aca.2018.07.027
0003-2670/

interest [1,2]. Because it can provide more precise measurements by normalizing variations in environmental changes, such an approach has been successfully coupled with various signal transduction techniques such as fluorescence, electrochemistry, and surface enhanced Raman scattering (SERS) for detecting different biomarkers, including proteins [3,4], DNA [5,6] and small molecules [7,8]. Among these methodologies, the electrochemical ratiometric sensors (ERSs) are of enormous interest because of the outstanding superiorities of the electrochemical technique in terms of high sensitivity, rapid detection, low cost, as well as ease of miniaturization. The common principle of the ERSs utilizes the signal ratio of two electroactive labels synchronously for detecting one given target with improved robustness and reproducibility [3,5]. Because the ERSs possess a unique self-referencing capability to the environmental variances, they therefore hold great promise for further improving the detection accuracy and precision for complex samples. The ERSs can be classified into two categories: the dual- and single-channel electrochemical ratiometric sensors [9]. The dual-channel electrochemical ratiometric sensors (DCERSs), in which two electroactive labels are confined on two independent interfaces, are not suitable for clinical diagnosis because of the difficulty of enabling the two interrogated interfaces in close conditions and the non-negligible errors in quantitative detection [8,10]. Given the inadequacies of DCERSs, the single-channel electrochemical ratiometric sensors (SCERSs) adopt an identical surface for the confinement of the two electroactive labels, which further improves the reproducibility of the sensors [9,11,12]. With the intrinsic advantages of simplicity and minor error in comparison with the DCERSs, the SCERSs have intrigued increasing interest in biodetections.

Single nucleotide polymorphisms (SNPs) first reported in 1994 were defined as polymorphisms of the genomic sequences caused by variations of single nucleotide (A, G, C, T) in forms of transition, transversion, deletion and insertion [13–15]. According to the human genome project, single nucleotide variations can occur once every 100–300 bases [16] and have shown to be crucial indexes to Alzheimer's disease, Parkinson's disease, diabetes and so on [17–19]. It is therefore important to develop methods for efficient detection of SNPs. Previous approaches for detecting SNPs can be principally categorized into enzyme-aided strategies and hybridization-based approaches [20]. The enzyme-aided strategies, such as real time polymerase chain reaction (PCR) assays [21,22], ligase detection reaction (LDR) [23,24] and sequence cleavage by endonuclease [25,26], are time-consuming and labor-intensive. On the other hand, the hybridization-based approach for quantifying SNPs utilizes the hybridization difference between the perfectly matched and single-nucleotide mismatched target to distinguish the slight thermodynamic stability difference between two sequences [27,28]. However, stringent assay conditions (e.g., precise control of hybridization temperature, pH and ionic strength) are commonly required to improve the capacity of discriminating single-base-mismatch in the hybridization-based approaches. According to previous reports, molecular beacons have higher specificity for gene recognition compared with linear DNA probes due to their intrinsic structural constraints [29–31], and their specificity and stability have been further improved by a new molecular beacon structure of triple helix complex (THC) reported by Tan group [32–34]. The THC consists of a hairpin structure probe and a single stranded DNA (ssDNA) and is formed by the hybridization of the two terminal segments of the ssDNA to the middle region of the hairpin, resulting in the unfolding of the original hairpin and the formation of a fresh molecular beacon structure with triple helix stem. The THC can exist in room temperature and simple buffer with good stability. Importantly, the THC shows improved specificity and binding capability to the target sequences than the double helix molecular beacons [33,35], making them hold great potential for discriminating SNPs.

By taking the advantages of the THC, we report herein an amplified electrochemical ratiometric sensor for detecting SNP with a significantly enhanced discrimination factor, using the mutant p53 gene (mp53, mutation of G to A at codon 175, exon 5 [29]) as the model target. The mutations of the tumor suppressor p53 gene have proved to be related to 50% of all malignant tumors [36], it is thus of great significance to monitor the mp53 gene for clinical diagnosis purposes. In our strategy, the addition of the mp53 causes the change of the current response ratio of two electroactive labels, ferrocene (Fc) and methylene blue (MB), which are conjugated to the THC probes on the sensor surface. In addition, such a change of ratio is significantly amplified by incorporating the toehold-mediated strand displacement reaction (TSDR)-aided recycling of the target mp53 gene, resulting in sensitive electrochemical detection of mp53 down to the sub-femtomolar level. Moreover, with the high specificity of the THC probes and TSDR, a significantly enhanced SNP discrimination factor (c.a. 41.7) is obtained for the mp53 against the wild p53 gene.

2. Experimental section

2.1. Reagents and materials

6-mercapto-1-hexanol (MCH), tris(2-carboxyethyl)phoshine hydrochloride (TCEP), sodium perchlorate monohydrate ($NaClO_4 \cdot H_2O$) and 4-(2-hydroxyethyl) piperazine-1 ethanesulfonic acid sodium salt (HEPES) were supplied by Sigma-Aldrich (St. Louis, MO, USA). The DNA sequences listed below in Table 1 were HPLC-purified and synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China). Other chemicals of analytical reagent grade and ultrapure water (resistivity of 18 MΩ-cm) were used for all experiments.

2.2. Pretreatment of the gold electrode (AuE)

The AuEs (3.0 mm in diameter) were pretreated according to our previous protocol [37]. In brief, the AuEs were first immersed in freshly prepared piranha solution (consisting of 98% H_2SO_4 and 30% H_2O_2 with a volume ratio of 3:1) for 0.5 h, followed by washing with ultrapure water. Then, two polishing steps of the AuEs with 0.3 and 0.05 μm alumina slurries for 5 min, respectively, were performed. The electrochemical cleaning step was further performed in the potential range from −0.3 V to +1.55 V in 0.5 M H_2SO_4 until a stable and remarkable voltammetric peak was observed. Finally, the pretreated AuEs were dried with nitrogen and used for sensor preparation.

2.3. Fabrication of the sensors

The THC was firstly obtained by mixing MB-HP (1.0 μM) with Fc-CP (1.2 μM) in 10 mM phosphate buffer (20 mM NaCl, 2.5 mM $MgCl_2$, pH 6.2), followed by incubating for 2 h at 25 °C. Then, the THC was incubated with 10 mM TCEP for 60 min at 25 °C in a microcentrifuge tube, followed by being diluted to a final concentration of 0.4 μM. Subsequently, 10 μL of the THC was cast onto the pretreated AuE for 2 h at 25 °C in the dark. After washing by phosphate buffer, the modified electrode (THC/AuE) was passivated with 1 mM MCH solution for 2 h, followed by another washing step to obtain the sensor (THC/MCH/AuE). After that, the THC/MCH/AuEs were further incubated with various concentrations of the mp53 in the presence of AS-HP (1.0 μM) at 25 °C for 100 min. This was followed by washing the sensors with buffer and electrochemical measurements were further performed.

Please cite this article in press as: B. Dou, et al., Electrochemical screening of single nucleotide polymorphisms with significantly enhanced discrimination factor by an amplified ratiometric sensor, Analytica Chimica Acta (2018), https://doi.org/10.1016/j.aca.2018.07.027