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Identification of glutathione by voltammetric analysis with rolling circle amplification

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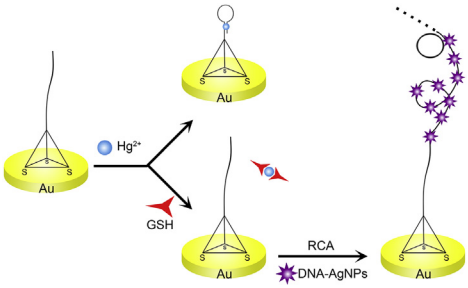
HIGHLIGHTS

- A novel voltammetric biosensor for glutathione detection is fabricated.
- Coordination of Hg^{2+} to glutathione is more favorable than that of T-Hg^{2+}-T.
- Rolling circle amplification product recruits AgNPs for stripping current response.
- The biosensor has a detection limit of 0.1 pM.
- This biosensor performs satisfactorily in real samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Glutathione (GSH), a common tripeptide, plays an essential role in a variety of cellular functions. GSH level is reported to be closely related to human health. In this study, we fabricate an ultrasensitive electrochemical biosensor for GSH quantification. DNA probes are firstly modified on the electrode surface and thymine-Hg^{2+}-thymine is formed. Since GSH is able to chelate Hg^{2+} from the DNA mismatched sites effectively, which leads to DNA structural switching from hairpin to linear strand, rolling circle amplification (RCA) could be initiated with the released linear primer probe. The RCA product with multiple repeating sequences further captures numerous DNA modified silver nanoparticles (AgNPs) by the hybridization of complementary sequences. Stripping voltammetric responses of AgNPs are then detected to reveal GSH concentration. The linear detection range is from 0.1 pM to 10 nM and the limit of detection is 0.1 pM, which is lower than most current analytical methods. This method is also highly selective and functions well against a series of interferents. Additionally, the proposed method has been successfully utilized in human serum samples, which shows fairly good potential in clinical applications.

1. Introduction

Sulfhydryl groups are naturally present in animals, plants and microscopic forms of life, which have a variety of functions like sequestering metal ions and quenching reactive oxygen species [1,2]. Glutathione (GSH), also named as L-γ-glutamyl-L-cysteinylglycine, is one of the most abundant biothiols and endogenous antioxidants, which plays essential roles in cellular defense against inimical heavy metals and energetic free radicals [3,4]. Numerous studies have established relationships between the concentrations of this tripeptide in cells or blood plasma with human health conditions. Blood GSH level is reported to be able to reflect the redox status and can be considered as a useful disease indicator [5]. Abnormal levels of GSH are closely associated with

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different human disorders such as heart disease, liver damage, age-related hearing loss, Parkinson's disease, and cancer [6–10]. Therefore, development of sensitive methods to determine the presence or concentration of GSH is urgently needed [11].

Despite the availability of different techniques for GSH detection, including high-performance liquid chromatography (HPLC) [12], mass spectrometry [13], and fluorescence-based methodology [14,15], several disadvantages are suffered, which may limit their wide applications. These methods may require sophisticated equipment, expensive fluorescence probe and well-trained personnel. On the contrary, electrochemistry-based methodology benefits from merits of rapid response, low cost, convenient operation, high sensitivity and selectivity, which is suited to the detection of GSH [16–20]. For example, Oztekin et al. prepared a poly-*m*-aminophenol modified glassy carbon electrode for electroanalytical determination of GSH [21]. Ru et al. employed multi-walled carbon nanotubes/ubiquinone/ionic liquid nanocomposite as a transducer for GSH sensing [22]. Zhad et al. developed an electrochemical "signal-on" sensor based on the coordination of GSH and mercury ions (Hg^{2+}) that are bound to DNA probe on electrode surface [23]. Areias et al. reported a voltammetric sensor in which GSH forms a 1:1 complex compound with copper ions (Cu^{2+}) for characteristic electrochemical oxidation [24].

In this report, we present a novel electrochemical approach for GSH analysis with simple modification design and high sensitivity. Rolling circle amplification (RCA) is initiated after DNA structural switching on the electrode surface, which is induced by GSH mediated Hg^{2+} displacement from thymine-Hg^{2+}-thymine. Further attached silver nanoparticles (AgNPs) by DNA hybridization provide significant silver stripping signals, which not only reveal electrochemical behaviors during different modification stages, but also provide quantitative information of GSH concentration.

2. Experimental

2.1. Materials and chemicals

GSH, trisodium citrate, silver nitrate ($AgNO_3$), mercury nitrate ($Hg(NO_3)_2$), sodium borohydride ($NaBH_4$), tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and hexaammineruthenium(III) chloride ($[Ru(NH_3)_6]^{3+}$) were ordered from Sigma (USA). Phi29 DNA polymerase and T4 DNA ligase were purchased from New England Biolabs Ltd. (Beijing, China). Human serum samples were supplied by local hospital (Suzhou, China). The other reagents were of analytical grade and were used as received. All solutions were prepared with water that was purified from a Millipore system. DNA oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences were listed in Table 1. The underlined bases of tetrahedron A constitute thymine-thymine mismatches.

2.2. Instrumentation

All electrochemical experiments were carried out on a CHI 660D electrochemical workstation (CHI instruments, Shanghai, China). Traditional three electrode system was applied, which contained platinum wire auxiliary electrode, Ag/AgCl reference electrode and gold working electrode. After the modification steps of working electrode, linear sweep voltammetry (LSV) experiments were performed in the electrolyte of 0.1 M KCl with the scan rate of 0.1 V s^{-1}. Chronocoulometry (CC) experiments were performed in 10 mM Tris-HCl buffer solution containing 50 μM $[Ru(NH_3)_6]^{3+}$ with the pulse period of 250 ms.

2.3. DNA tetrahedron formation

DNA tetrahedron that was used to modify the electrode was prepared as follows. Four single-stranded DNA solutions (tetrahedron A, B, C, and D) were firstly prepared in 10 mM Tris-HCl buffer with 10 mM TCEP, 50 mM $MgCl_2$ (pH 8.0). The final concentrations were 4 μM. Next, the four solutions were blended with the ratio of 1: 1: 1: 1. The mixture (DNA tetrahedron solution) was then heated to 95 °C for 2 min, which was cooled to room temperature for further use.

2.4. Electrode pretreatment and modification

The gold electrode (2 mm) was incubated with piranha solution (98% H_2SO_4: 30% H_2O_2 = 3: 1) for 5 min (*Caution: Piranha solution reacts violently with organic solvents and should be handled with great care!*). Next, it was polished to a mirror-like surface with P5000 sand paper and then 1, 0.3, 0.05 μm alumina slurry, respectively. The electrode was cleaned by ultrasonication for 5 min in ethanol and then in double-distilled water. Subsequently, it was immersed in 50% HNO_3 for 0.5 h and then electrochemically cleaned with 0.5 M H_2SO_4 for 20 cycles to remove any remaining impurities. After that, the electrode was dried with nitrogen and was incubated with DNA tetrahedron solution for 8 h.

2.5. Preparation of DNA-AgNPs conjugates

Synthesis of bare AgNPs was carried out by the borohydride reduction of $AgNO_3$. Briefly, 0.25 mM $AgNO_3$ and trisodium citrate solution was prepared, which was then blended with 10 mM $NaBH_4$ solution. The volumes were 100 mL and 3 mL, respectively. The mixture was under violent stirring for 0.5 h and then left to sit overnight in the dark. Yellow colored and transparent AgNPs solution was thus synthesized, which was then purified by three cycles of centrifugation at 12000*g* for 0.5 h. The as-prepared AgNPs solution was further interacted with 10 μM DNA signal probe for 24 h to achieve silver-amino binding. Finally, DNA-AgNPs conjugates were purified by centrifugation procedure.

2.6. Electrochemical detection of GSH

For the formation of hairpin structure on top of DNA tetrahedron at the electrode surface, the electrode was incubated with 15 nM Hg^{2+} for 1 h. Hg^{2+} was dissolved in H_2O directly and pH was

Table 1
DNA sequences used in this work.

Name	Sequence (from 5′ to 3′)
Tetrahedron A	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTACCGTTTGGGTTTTTAATCCCTATAAATACCCTAAC
Tetrahedron B	SH-$(CH_2)_6$-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
Tetrahedron C	SH-$(CH_2)_6$-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
Tetrahedron D	SH-$(CH_2)_6$-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
Padlock probe	phosphoryl-TTATAGGGATTCTCTATCTGTTAGGGTAT
Signal probe	NH_2-$(CH_2)_6$-GATTCTCTATCT