Analytica Chimica Acta 934 (2016) 59–65

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Amplified electrochemical detection of nucleic acid hybridization via selective preconcentration of unmodified gold nanoparticles

Yuan Li [a], Rui Tian [a], Xingwang Zheng [a, **], Rongfu Huang [b, *]

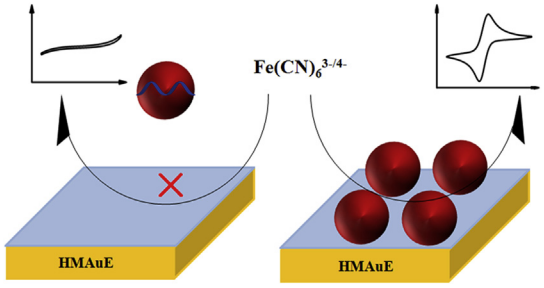
[a] Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, China

[b] College of Materials, Chemistry and Chemical Engineering, Chengdu University of Technology, Chengdu 610059, China

HIGHLIGHTS

- Differential adsorption ability for single-/double-stranded nucleic acid onto U-AuNP.
- Selective preconcentration of U-AuNPs to mediate amperometric signal.
- Amplified amperometric signal and low background current.
- 10^5 times improved sensitivity compared to colorimetric method.
- Novel sensing platform with general feasibility.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form
21 June 2016
Accepted 21 June 2016
Available online 28 June 2016

Keywords:
MicroRNA
DNA probe
Unmodified gold nanoparticle
Electrochemical method
Preconcentration effect

ABSTRACT

The common drawback of optical methods for rapid detection of nucleic acid by exploiting the differential affinity of single-/double-stranded nucleic acids for unmodified gold nanoparticles (AuNPs) is its relatively low sensitivity. In this article, on the basis of selective preconcentration of AuNPs unprotected by single-stranded DNA (ssDNA) binding, a novel electrochemical strategy for nucleic acid sequence identification assay has been developed. Through detecting the redox signal mediated by AuNPs on 1, 6-hexanedithiol blocked gold electrode, the proposed method is able to ensure substantial signal amplification and a low background current. This strategy is demonstrated for quantitative analysis of the target microRNA (let-7a) in human breast adenocarcinoma cells, and a detection limit of 16 fM is readily achieved with desirable specificity and sensitivity. These results indicate that the selective preconcentration of AuNPs for electrochemical signal readout can offer a promising platform for the detection of specific nucleic acid sequence.

1. Introduction

The interactions of unmodified gold nanoparticles (AuNPs) with nucleic acid have been widely studied for years [1,2]. It was found that single-stranded DNA (ssDNA) and DNA complex have different propensities to adsorb onto AuNP surface in colloidal solution. In the mixture solution of ssDNA and AuNPs, ssDNA is flexible and can partially uncoil its bases. The attractive van der Waals forces between the bases and the AuNPs are sufficient to overcome the repulsion between the negative charged phosphate backbone of ssDNA and the adsorbed citrate ions on AuNPs. Then, ssDNA can stick to the surface of AuNP. The same mechanism, however, is not

* Corresponding author.
** Corresponding author.
E-mail addresses: zhengxw@snnu.edu.cn (X. Zheng), huangrongfu13@cdut.cn (R. Huang).

http://dx.doi.org/10.1016/j.aca.2016.06.035
0003-2670/

operative with DNA complex (e.g. double-stranded DNA, dsDNA) because of their stronger repulsion and rigid structure, which do not permit the uncoiling needed to expose the bases [3]. Therefore, many researchers used the disparity in adsorption ability for ssDNA and DNA complex on AuNP to design a rapid identification assay for nucleic acid sequence [4–7], ions [8] and proteins [9,10]. Compared with the nucleic acid sensing system involving in the conjugation of nucleic acid sequence with nanoparticles, the application of AuNPs as the sensing element owned the following advantages: Firstly, the immobilization-free and label-free DNA probes avoided controlling over the probe density and orientation, which greatly simplified the task in the procedures of sensor preparation. Secondly, the recognition reaction of probe ssDNA with its target in homogeneous solution makes it easy-going with high reaction efficiency; According to the sensing mechanism, thirdly, it is proven that the application of nanosized surface as the sensing element to reflect the nanoscaled conformation change ensures good specificity and high selectivity.

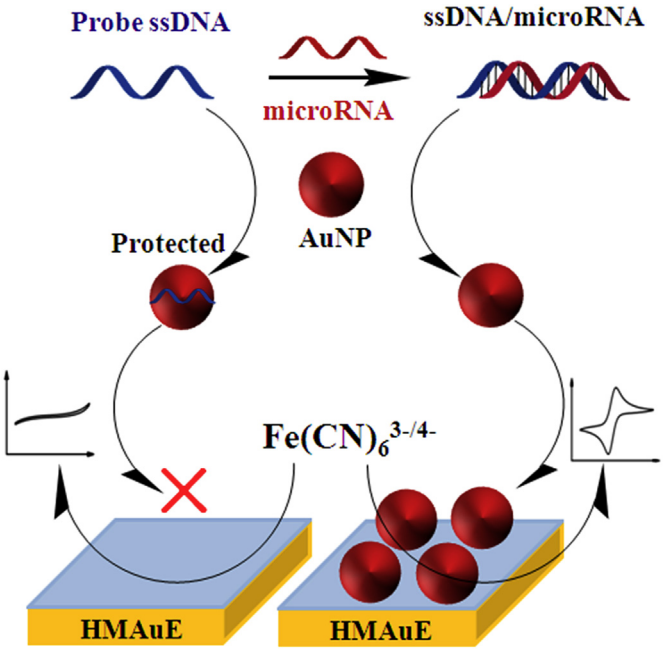
Up to now, colorimetric [3,11] and fluorescent [12] method were developed for rapid detection of nucleic acid using the disparity in adsorption ability for ssDNA and DNA complex on AuNP, which were based on the color changes of AuNPs or fluorescence quenching effect of AuNPs to fluorophore tagged on probe ssDNA. However, two types of methods both suffered from the poor sensitivity. Ordinarily, a nanomolar detection limit was often achieved [3]. Thus, some signal amplification schemes were introduced to couple with the proposed sensing strategy, such as polymerase chain reaction (PCR) [13], hybridization chain reaction (HCR) [14], self-catalytic AuNPs size enlarging scheme [15] and target-induced strand-cycle reaction [16], etc. However, the complicated procedures for signal amplification limited their practical application. Thus, the design of a new sensing strategy using unmodified AuNPs is fascinating.

Electrochemistry offers great advantages because it provides rapid, simple, and low-cost detection. Introduction of the metallic nanoparticles with unique chemical and physical properties into electrochemical (bio) sensors will greatly improved sensitivity, selectivity and dynamic range in comparison with conventional sensors. In this study, a novel electrochemical nucleic acid sensing strategy through the preconcentration of unprotected AuNPs by ssDNA binding to mediate amperometric signal was developed, and a microRNA sequence was selected as the model target to demonstrate our idea. As we know, microRNAs are short endogenous noncoding RNAs which play important roles in gene regulation and cell function. Moreover, specific changes in microRNA expression patterns are associated with various diseases, such as cancer. Therefore, microRNA detection is crucial for early diagnosis of diseases and discovery of new targets for drugs. As illustrated in Scheme 1, adsorption of probe ssDNA on the surface of AuNP led to the formation of ssDNA protected AuNPs. These ssDNA protected-AuNPs could not be efficiently preconcentrated on a 1, 6-hexanedithiol modified gold electrode, producing negligible amperometric signal in the solution of $Fe(CN)_6^{3-}$. After experienced a hybridization reaction, in contrast, the formation of ssDNA/microRNA heteroduplex cannot effectively bind to the surface of AuNPs. Then, the AuNPs in free state can be selectively preconcentrated on electrode surface, producing significantly enhanced amperometric signal.

2. Material and method

2.1. Materials and reagents

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$), 1, 6-hexanedithiol, and trisodium citrate dihydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). RNase-free water was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). HPLC-purified microRNA and all synthetic oligonucleotides were obtained from Shanghai Sangon Biotech (Shanghai, China). The sequences of all microRNA and DNA used were available in Table S1. Trizol Reagent was purchased from Invitrogen (Beijing, China). Chloroform and isopropyl alcohol were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. All other reagents were analytical grade and solutions were prepared using ultrapure water (sterile Millipore water, 18.3 MΩ cm). The solution used in this work were as follows: DNA stock solutions buffer (10 mM Tris-HCl, 100 mM NaCl, pH 7.4), 5 mM $Fe(CN)_6^{3-}$ in 0.1 M phosphate buffer solution with 0.1 M KCl (PBS, pH 7.4).



Scheme 1. Cartoon representation of the proposed method for electrochemical detection of target nucleic acid sequence.

2.2. Instruments

Electrochemical measurements were performed at room temperature by using CHI 660C electrochemical analyzer (Chenhua Inc., Shanghai, China). A three-electrode cell (5 mL with a diameter of 25 mm) consisting of modified gold working electrode, Ag/AgCl reference electrode, and platinum counter electrode was used for all electrochemical measurements. The potential window in cyclic voltammograms scan ranged from −0.3 to 0.6 V (versus Ag/AgCl) in 3 mL of $Fe(CN)_6^{3-}$ solution with scan rate of 100 mV s^{-1}. Transmission Electron Microscopy (TEM) image of the AuNPs was performed using a JEM-2100 TEM (Hitachi, Japan). Characterizations of the interaction between AuNPs and ssDNA or dsDNA were recorded with a UV–vis spectrophotometer (TU1901, China). High speed centrifuge (5804 R, Eppendorf) was used for extraction of microRNA. Field Emission Scanning Electron Microscopy (FESEM, Hitachi SU8020, Japan) was used to characterize the preconcentration of AuNPs on 1, 6-hexanedithiol modified electrode.

2.3. Synthesis of AuNPs and preparation of 1, 6-hexanedithiol modified electrode

AuNPs with a diameter of 13 nm were prepared by the citrate