An electrochemical signal transduction amplification strategy for ultrasensitive detection of ascorbic acid

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ABSTRACT

A high sensitive electrochemical signal transduction amplification strategy was constructed based on the integration of the target-triggered silver deposition and the high sensitive stripping voltammetry detection. The Au-nanoparticles (AuNPs), multi-walled carbon nanotubes (MWNTs) and polyethyleneimine (PEI) modified glassy carbon electrode (AuNPs/MWNTs/PEI/GCE) was prepared and used as the working electrode which could also amplify the electrochemical signal dramatically due to the excellent electronic property of the electrode and the Au-nanoparticles induced silver deposition reaction. This strategy was applied to the high sensitive ascorbic acid (AA) determination and a detection limit as low as 15 fM was achieved, which was 6–9 orders of magnitude more sensitive than other reported methods for AA determination. The proposed method has good selectivity and anti-interference ability, which could be applied to AA detection in real complex samples.

1. Introduction

Ascorbic acid (AA) is one of the essential vitamins known as vitamin C, which is extensively used in food processing and pharmaceuticals due to its antioxidant peculiarity. It has been demonstrated that AA play critical roles in various physiological and pathological processes [1,2]. The deficiency of AA may result in cataract scurvy, atherosclerosis and other disease. It is therefore of great importance to developing facile analytical methods for high sensitive and selective measurement of AA. Several methods have been developed for ascorbic acid detection, including electrochemistry [3–6], chromatography [7,8], colorimetry [9–11], fluorescence spectroscopy [12–14] and so on. Among these methods, the electrochemical approaches are convenient and suitable for AA detection because of their unique features, such as high sensitivity, low cost, and ease of miniaturization. Unfortunately, although AA is electrochemically active, its sluggish electron-transfer kinetics and severe electrode fouling by its oxidized product present great barriers for electrochemical measurement of AA selectively [15,16]. Great effort has been made to solve this problem.

Nanomaterials exhibit unique electronic properties, such as large specific surface area, good conductivity and biocompatibility, which can greatly enhance the performance of electrochemical biosensors [17–19]. As a result, carbon nanotube, graphene, Au nanoparticle (AuNP) and other metal nanoparticles were extensively used for electrode modification, and the resulting modified electrodes always exhibit

excellent electronic property. Different nanomaterial modified electrodes were prepared and these electrodes could realize the fast electron-transfer kinetics of AA [20–23]. These chemically modified electrodes could be applied for electrochemically detection of AA directly, and the simultaneous electrochemical detection of AA and other substances such as dopamine and uric acid could be also realized. However, many of these methods do not meet the growing requirement for sensitive determination of AA especially in the sample related to its biologic function [24] and treatment of cancer [25]. Therefore, the further development of simple and high sensitivity for detecting AA is highly desirable.

Anodic stripping voltammetry (ASV) was a very high sensitive electrochemical detection technique, which is widely used in determination of heavy metals in liquid [26–28]. In this study, an ultrahigh sensitive electrochemical strategy for AA determination was proposed based on the integration of the target-triggered silver deposition, the high sensitive metal stripping voltammetric detection and AuNPs induced silver deposition. The presence of AA in detection solution could lead to a rapid silver deposition process on the surface of the AuNPs/MWNTs/PEI/GCE in the presence of Ag^+, which could dramatically amplify the electrochemical signal and enhance the detection sensitivity. The electrochemical signal was obtained by anodic stripping analysis of the deposited silver in KCl solution. A very good linear dependence between the ASV signal of deposited Ag and AA concentration is attained and the detection limit is determined to be 15 fM. The present work not only provides a simple platform for ultrasensitive AA detection, which has a broad prospect in clinical diagnosis applications, but also initiates a new electrochemical signal amplification strategy.

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2. Experimental

2.1. Chemicals and apparatus

Multi-walled carbon nanotubes (MWNTs, 10–20 μm in length, 50 nm in outer diameter and 10–15 nm in inner diameter) were purchased from Chengdu Organic Chemicals Co. Ltd. Chinese Academy of Sciences (Chengdu, China). The MWNTs were carboxylated by ultrasonicating in concentrated nitric acid and then washed with plenty of water, finally the carboxylated MWNTs were redispersed in water before use. Polyethylenimine (PEI), AA, silver nitrate and other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

All electrochemical measurements were performed on a CHI 660d electrochemical workstation (Shanghai Chenhua Co., China). Platinum wire and saturated calomel electrode (SCE) worked as counter and reference electrode, respectively. The bare glassy carbon electrode (GCE, 3 mm diameter) or a modified GCE was employed as a working electrode. The scanning electron microscope (SEM, FEI. quanta 200, America) was used to characterization the surface morphology of the working electrodes.

2.2. The modification of the GCE

GCE was polished with 0.3 and 0.05 μm alumina powder sequentially, and washed ultrasonically in ethanol and ultrapure water. The MWNTs and PEI modified GCE (MWNTs/PEI/GCE) was prepared by dropping a 5 μL 1 mg mL^{-1} PEI aqueous solution containing 1 mg mL^{-1} carboxylated MWNTs on the surface of the GCE, and then dried under room temperature. The MWNTs/PEI/GCE was immersed in 5 mM HAuCl4 solution which containing 0.1 M KCl and then treated by applying −0.2 V (vs SCE) for 60 s for electrochemical deposition of AuNPs [29], and then the AuNPs/MWNTs/PEI/GCE was prepared. The modified electrode was stored at 4 °C before use. For comparison, the AuNPs/GCE and MWNTs/PEI/GCE were prepared by deposition AuNPs or dropping MWNTs/PEI solution on the surface of the GCE, and then dried under room temperature.

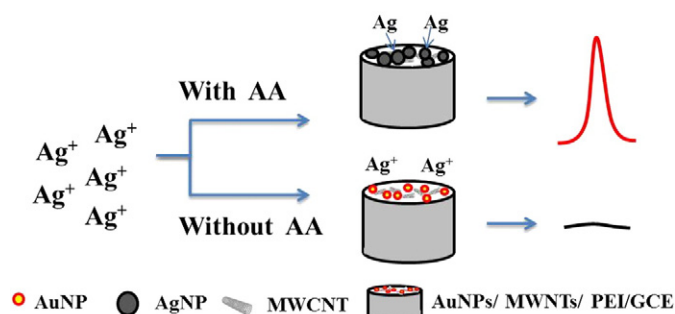## 2.3. Electrochemical detection of AA

Different concentrations of AA (500 μL) were added into the same volume detection solution, which consisted of 2 mM AgNO$_3$ in diethanolamine solution (DEA; 0.2 M). The AuNPs/MWNTs/PEI/GCE was immersed in the resulting mixture and incubated at 37 °C in dark with continuous magnetic stirring for settled time. Then the working electrode was washed with plenty of water and immersed in 1 M KCl solution for electrochemical detection with a Pt wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode. Linear sweep voltammetric (LSV) measurement from −0.1 to 0.4 V (vs SCE) with a scanning rate of 100 mV s^{-1} was performed for the electrochemical detection.

To test the selectivity of this method, 0.1 nM of ascorbic acid and 20 μM to 2 mM (2×10^5 to 2×10^7 times of ascorbic acid) of foreign ions and molecules were used for comparison. As for pharmaceuticals product detection, two different ascorbic acid products, purchased from local drugstore were used for sample detection. Firstly, one tablet of the each product was prepared in a sample solution after a series of steps of crushing, grinding, dissolution and centrifugation. Then, the as-prepared sample solutions were detected by the standard addition method.

3. Result and discussion

3.1. The mechanism of the electrochemical signal transduction amplification strategy for ascorbic acid detection

The principle of the electrochemical signal transduction amplification strategy for ascorbic acid detection was showed in Scheme 1. The

Scheme 1. Working principle of the electrochemical signal transduction sensing strategy for ascorbic acid detection. The presence of AA could reduce Ag$^+$ to Ag^0 on the surface of the AuNPs/MWNTs/PEI/GCE, resulting in a large stripping voltammetric electrochemical signal.

presence of AA in the detection solution could reduce Ag$^+$ to Ag^0 on the surface of the AuNPs/MWNTs/PEI/GCE rapidly due to the target-triggered silver deposition and the AuNPs induced silver deposition reaction. As a result the electrochemical signal of AA could be accumulated on the surface of the working electrode by means of silver deposition, which amplified the electrochemical signal dramatically. Thus a very large stripping voltammetric electrochemical signal could be obtained by anodic stripping analysis of the working electrode in KCl solution. Whereas the absence of the AA in the detection solution couldn't react with Ag$^+$, thus the stripping voltammetric electrochemical signal of Ag^0 was negligible.

3.2. The availability of this strategy for ascorbic acid detection

To investigate the availability of the signal transduction strategy, the AuNPs/MWNTs/PEI/GCE was immersed in different solutions for 20 min and the resulting LSV signals were showed in Fig. 1A. Only in the presence of Ag$^+$ and AA in the detection solution could lead to a large electrochemical signal, and the absence of Ag$^+$ or AA in the detection solution could only lead to negligible background electrochemical signals. The electrochemical signal obtained through this electrochemical signal transduction system could be related to the deposition of Ag$^+$ on the surface of the AuNPs/MWNTs/PEI/GCE which was triggered by AA in the detection solution and induced by AuNPs on the surface of working electrode, the absence of AA or Ag$^+$ in detection solution could block the signal transduction reaction and lead to a negligible background current.

3.3. The amplification effect of the AuNPs/MWNTs/PEI/GCE

The use of the AuNPs/MWNTs/PEI/GCE could amplify the detection signal dramatically. The LSV signal obtained from different electrodes was compared in Fig. 1B. The LSV signal of AuNPs/MWNTs/PEI/GCE was much larger than these signals obtained by other electrodes such as bare GCE, MWNTs/PEI/GCE, AuNPs/GCE and gold electrode, which proved that the AuNPs/MWNTs/PEI/GCE was beneficial to this electrochemical signal transduction system. Since the half-wave potential of AA is lower than Ag, AA can reduce Ag$^+$ to Ag^0 spontaneously. But this reduction process is too slow without the AuNP nucleation sites [30]. As a result the presence of AuNPs on the working electrode could greatly enhance the stripping signal, owing to the pronounced effect of AuNPs on silver deposition process coupled with the increased number of nucleation sites on the electrode. The presence of MWNTs and AuNPs could also improve the electrical conductivity and increase the surface area of the working electrode which benefit to the electrochemical detection as well. As a result the AuNPs/MWNTs/PEI/GCE was suitable for the electrochemical signal transduction strategy and the detection signal could be amplified greatly.