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Research paper

Single cytosine-based electrochemical biosensor for low-cost detection of silver ions

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ABSTRACT

The development of Ag^+ detection system with high sensitivity, selectivity and quantitatively accurate analysis is crucial because of the potential risk of Ag ion increasingly applied in various fields. As synthetic oligodeoxyribonucleotides are available to use, there have been few studies reported in the literature regarding techniques for electrochemical biosensors based on cytosine-enriched DNA used as the capture material of Ag ion. The high price and complex process for connecting NH_2-terminal to DNA make it expensive to fabricate the Ag detecting system. In this study, we investigated the Ag ion detection system with single-cytosine (SC) used in the place of DNA with a functional group. The proposed system can guarantee the effect of span increase, price reduction of detection system, and lower detection limit due to the increase of immobilization efficiency by using SC as a capture probe.

Three-electrode system was microfabricated on a glass wafer. SC was immobilized on the working electrode surface, and then this electrode was exposed to a solution with Ag ions and other SC for mismatching cytosine-Ag^+-cytosine. Before detecting Ag ion, cyclic voltammetry and energy-dispersive X-ray spectroscopy were used for the identification of stable immobilization of SC on the surface of the functionalized Au electrode. The electrochemical analysis for increasing Ag ion concentration and the Ag^+ selectivity of the SC probe were performed by square wave voltammetry. The resulted current increase is linear with the logarithmic concentration of Ag ion from 0.5 nM to 1 mM with ~20 pM of detection limit. The slope of in a target concentration range mentioned above are 9.14×10^{-2} per concentration decade with R^2 value of 0.9814. The reason why our system has a lower detection limit than the previous studies is because the bound each single Ag ion performs a path role for electron transfer. Moreover, the proposed electrochemical assay has good selectivity to other environmentally relevant metal ions.

1. Introduction

Silver nanoparticle is being strongly considered as special additive of antibacterial products in various industries as well as food, cosmetic, and medical industry owing to its antibacterial effect [1,2]. However, recently substantial research efforts have been paid to reveal that this ion is harmful to the human body [3–7]. This ion causes cytopathogenic effect because of the inactivation of sulfhydryl enzyme by coupling with various metabolites, resulting in changing the physical properties of this enzyme, thus depositing the protein. In particular, this ion infiltrates easily into human body owing to its strong oxidation potential, which may cause death. Therefore, it is of critical importance to develop the detection system of silver ion with high sensitivity, selectivity and quantitatively accurate analysis.

A variety of techniques, such as atomic absorption spectrometry [8,9], plasma atomic emission spectrometry [10], inductively coupled plasma-mass spectroscopy [11,12], and fluorescence spectroscopy [13–16], have been developed for the detection of silver ion. Although Traditional methods have been successfully applied to detect trace levels of silver, there are still many limitations in practical applications because experts for operating and deciphering these techniques are required, and results will be obtained relatively slow. Especially, atomic absorption spectrometry has limited range of measurement. Also, fluorescence spectroscopy is not suitable for on-site diagnoses owing to the issues on the quantitative analysis and the emission of toxic substances as result of the detection [17,18].

With the development of DNA synthesis technique, the synthesis of oligodeoxyribonucleotide has become very easy, and synthetic oligodeoxyribonucleotides containing artificial bases

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have been used to form metal-mediated base pairs. Watson-Crick-type base pair hydrogen bonding in natural DNA is gradually replaced with metal-base bond in many areas [19]. In particular, oligodeoxyribonucleotides, proved to be a capture materials, have been used for the detection of Ag ion in sensing nanoparticles from environmental aspect. Moreover, there are a few challenges to apply oligodeoxyribonucleotides on electrochemical sensor [20,21]. Although these studies had shown the stable characteristics by using many advantages of electrochemical method such as simplicity, fast detection time, detection capacity at a low concentration, and cost effectiveness, this method still need to evolve for pertinence to point-of-care detection. In the above mentioned reports, there are challenge to apply cytosine(C)-enriched DNA as the capture material of Ag ion for formation of stable cytosine-Ag^+-cytosine complex. For the immobilization of this DNA on the electrode surface, the NH_2-terminal was connected to DNA, leading to high detection limit as well as increasing the electrode fabrication cost due to complex and expensive connection process.

In this study, we introduce the advanced Ag ion analysis using the Ag^+ mismatch-interaction of with single-cytosine (SC) instead of DNA with functional group as a probe. To show the improved characteristics of proposed strategy, the miniaturized and microfabricated three-electrode system was integrated on glass substrate. Process parameters for each fabrication process were experimentally optimized to obtain a high performance for the proposed system. The proposed strategy, compared to cytosine-enriched DNA-based sensors, shows higher immobilization efficiency with lower electrode fabrication costs due to using NH_2-terminal of SC, widely measurable range (span) and especially low detection limit (LOD) which was 1000 times higher in sensitivity.

2. Experimental

2.1. Materials

3-Mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, $C_6H_{17}N_3$), potassium chloride (KCl), potassium ferricyanide ($K_3[Fe(CN)_6]$), tris-EDTA (TE, pH 7.6) buffer, SC, sulfuric acid (98%), 30 wt% hydrogen peroxide, buffered oxide etchant (BOE), calcium chloride ($CaCl_2$) and silver nitrate ($AgNO_3$) were purchased from Sigma-Aldrich (St. Louis, USA). The six groups of metal ions (Li^+, Na^+, Hg^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Al^{3+}) and SC were prepared for the selectivity analysis (Sigma-Aldrich). N-Hydroxy-succinimide ester (NHS) was obtained from Alfa Aesar (Ward Hill, USA), and phosphate-buffered saline (PBS, pH 7.4) was bought from GIBCO (Carlsbad, USA). 4 inch Pyrex glass wafer purchased from 4 Science (Seoul, Korea). $CaCl_2$-TE buffer solution consisted of 1 M $CaCl_2$ in TE buffer solution. Ag ions were generated by dissolving silver nitrate in deionized (DI) water for 2 h, and the six types of metal ions for the selectivity analysis were prepared in the same way. A 40 mM MPA solution was prepared in a mixture of 99% ethanol and DI water. A 75 mM solution of EDC and 30 mM NHS solution was prepared as crosslinker. Electrochemical measurement was performed in a 3 M KCl solution containing 10 mM $K_3[Fe(CN)_6]$. Other chemicals and materials were used without further purification.

2.2. Characterization

The morphology of the Ag-bound electrode was performed by scanning electron microscopy (SEM, S-4300, Hitachi Ltd., Japan), and the chemical compositions of this electrode were observed by energy dispersive x-ray analysis (EDX) performed with EX-200 spectrometer coupled with the microscope. The electrochemical analysis was investigated using a 263A Perkin Elmer potentiostat/galvanostat (Princeton Applied Research, USA). To record the electrochemical measurements, the following instrument parameters were used: cyclic voltammograms (scan rate = 100 mV/s), square-wave voltammograms (SWV) (step potential = 1 mV, square-wave amplitude = 2 mV, frequency = 10 kHz, scan rate = 50 mV/s).

2.3. Integration of three electrode system

A three-electrode system, consisting of a gold working electrode of 5 mm diameter, a gold counter electrode, and a silver chloride reference electrode (Ag/AgCl), was microfabricated on a 4-inch pyrex glass wafer. To decidedly define the electrode area and avoid the biomolecule immobilization at undesirable region, the entire substrate was passivated with a 200 nm silicon dioxide (SiO_2) and 300 nm nitride layer by the plasma-enhanced chemical vapor deposition. Then, the passivated layers were etched utilizing a two-step process including the nitride etching by the reactive ion etching (RIE) followed by the silicon dioxide etching in buffered oxide etchant solution.

2.4. Fabrication of biosensor platform

After cleaning with piranha solution (H_2SO_4:H_2O_2 = 3:1) for 15 min, self-assembled monolayers (SAM) were formed on the gold surface by exposing 40 mM MPA solution for overnight. To activate the carboxyl groups, the SAM-modified gold electrode was immersed in a solution containing 75 mM EDC and 30 mM NHS for 3 h at room temperature. When the modified electrode was dipped in a $CaCl_2$-TE buffer solution containing SC for 2 h, the activated electrode formed an amide linkage with the amine groups of SCs. After washing with TE buffer solution and drying at room temperature, the prepared solutions of Ag ions and SC were simultaneously dropped onto SC-modified electrode for obtaining cytosine-Ag^+-cytosine forms. The resulting electrode was washed with DI water. Fig. 1 shows the schematic illustration of the three-electrode system and experimental procedure for producing the Ag ion-detecting device.

3. Results and discussion

The immobilization concentration of biomolecule on the electrode plays important role in determining the performance of sensor. To optimize the immobilization condition for the SC, acid-treated electrode was reacted with various concentrations ranging from 10 uM to 10 mM prior to the cyclic voltammetry (CV) measurements. Fig. S1 shows the electrochemical sensor response for various concentrations of SC. The increase of peak potential separation (ΔE) with the presence of SC reveals stable immobilization of SC on acid-treated electrode because SC impairs electron transfer capability. Cyclic voltammetry shows also that the peak current of SC-immobilized electrode decreased with increasing concentration and had an inflection point near 1 mM. Previously, Feng et al. reported that excessive concentrations of immobilized biomolecules lead to the increased steric hindrance of immunoreaction [22]. However, electrochemical response of our system at excessive concentrations showed saturation characteristics because cytosines have repulsive force for each other in neutral solution. We chose 1 mM as the optimal concentration of SC for efficient fabrication.

CV (Fig. S2) was performed for investigating the stable immobilization of SC and Ag^+ on the surface of the activated Au electrode. CV of each sample for a $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ redox couple was recorded in a 3 M KCl solution containing 10 mM $K_3[Fe(CN)_6]$