Oligonucleotide-Based Reusable Electrochemical Silver(I) Sensor and Its Optimization via Probe Packing Density

Kyoungsoo Kim, Je Hyun Bae, and Donghoon Han*

ABSTRACT: We report herein a selective, sensitive, and reusable electrochemical sensor for the detection of silver(I) ions. This sensor detects Ag⁺ through a structure-switching electrode-bound DNA by measuring the changes in the electron-transfer efficiency. A single-stranded DNA, featuring a methylene blue (MB)-tagged DNA hairpin structure, strategically provides selective binding for the silver-mediated coordination of cytosine–Ag⁺–cytosine complexes. The DNA-modified electrode produces a change in the electrochemical signal due to the redox current of the surface-confined MB tag. The “turn-on” signaling upon silver(I) ion binding could be attributed to a conformational change in the MB-tagged DNA from an open structure to a target-induced folding structure. Differential pulse voltammetry of the DNA-modified electrode showed that the MB reduction signal increased linearly with an increase in Ag⁺ concentrations in a range of 10−200 nM, with a detection limit of 10 nM. The structure-switching silver(I) ion sensor was amenable to regeneration by simply unfolding the electrode-bound MB-tagged DNA in 100 mM ethylenediaminetetraacetic acid, and it could be regenerated with no loss in signal gain upon subsequent silver(I) ion binding. We also demonstrated that by controlling the probe packing density on the electrode surface, the fabrication parameters can be varied to achieve optimal sensor performance.

1. INTRODUCTION

The development of simple, reliable, and practical sensors for the determination of environmental pollutants is a subject of considerable research. Of particular interest is the detection of silver, a hazardous pollutant that has been widely used in the medical, photographic, pharmaceutical, and electrical industries. It has been reported that large quantities of silver are released directly into the environment every year, which may lead to the pollution of ambient water, soil, and even food. The U.S. Environmental Protection Agency has declared that Ag⁺ concentrations higher than 1.5 nM are toxic to fish and microorganisms, and the maximum contamination level for Ag⁺ in drinking water has been set at 900 nM. Ag⁺ can interact with various metabolites and sulfhydryl enzymes, leading to disorders such as stomach distress, skin irritation, nervous system damage, and organ edema. Because of its adverse effects on human health, methods for the detection and quantification of Ag⁺ are in high demand. Many techniques are available, such as atomic absorption/emission spectroscopy and inductively coupled plasma mass spectrometry. However, these methods often require expensive and sophisticated instruments, time-consuming procedures, and complicated sample preparation. Recently, considerable effort has been made to develop Ag⁺ sensors using metal–organic frameworks, aggregation-induced emission nanoclusters, and colorimetric sensing strategies. However, there remain limitations associated with these methods regarding their practical application, such as insufficient selectivity and sensitivity, and interference from other competing metals. In these aspects, electrochemical methods are very attractive owing to their simple instrumentation and ease of miniaturization.

A particularly attractive alternative presented herein is molecular beacon-type electrochemical sensors for DNA based on an electroactive, labeled DNA stem-loop probe self-assembled onto a gold electrode. Despite advances in this field, there has been relatively little progress toward the development of practical, selective, reusable, “signal-on”-type electrochemical methods for the detection of Ag⁺ using the molecular beacon approach. Herein, we propose a simple and easily reusable electrochemical Ag⁺ sensor based on the conformational change in electrode-bound oligonucleotides, which involves a cytosine (C)-rich single-stranded DNA (ssDNA), taking advantage of specific C–Ag⁺–C complex formation. It has been reported that Ag⁺ can selectively bind between the cytosine bases of two strands and promote these

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C–C mismatches to form a stable C–Ag+–C coordination (Figure 1).22,23 The sensor employs a doubly labeled ssDNA

modified with a redox-active methylene blue (MB) moiety at its 3′-terminus as the reporter and a thiol functional group at its 5′-terminus as the anchor to the gold electrode surface. Among the available signaling redox moieties (most commonly MB or ferrocene), we have chosen MB for the redox-labeled DNA strand because it is far more stable than its ferrocene counterparts, particularly with regard to chemical stability in complex sample matrices.24 The electrode-bound MB-tagged DNA exhibits a voltammetric signal due to the electrochemical reaction of MB. In previous research, a sensing strategy in which the signal is “turned off” was reported.25 Using our approach, the probe DNA could instead generate electrochemical signals by the recognition of the analytes. The Ag+ sensing strategy is illustrated in Figure 1. In the absence of Ag+, the electrode-bound DNA is thought to be in an open conformation, and the distance between the MB redox tag and the electrode surface is expected to be relatively large. In the presence of Ag+, the electrode-bound DNA folds into a C–Ag+–C-mediated hairpin structure. This conformational change can improve the efficiency of electron transfer between the MB redox tag and the electrode surface, leading to an enhanced electrochemical signal. We also demonstrated the effects of the probe DNA packing density on the electrode surface to optimize the signaling properties of the sensor.

2. RESULTS AND DISCUSSION

Differential pulse voltammetry (DPV) is a voltammetric method that involves applying potential pulses to a linear ramp potential. It allows the detection of low concentrations of electroactive species compared to conventional sweep techniques, such as cyclic voltammetry (CV), because of the reduced contribution of non-Faradaic current. Therefore, DPV was used to assess the sensitivity and selectivity of the sensor under optimized detection conditions. Figure 2A shows the detailed changes in the current during DPV as a function of [Ag+]. When the concentration of Ag+ increased, the cathodic current signal increased because of the locked hairpin structure. This allowed the MB redox tag to be located closer, on average, to the electrode surface, providing an enhanced electrochemical signal from the MB/leucemethylene blue couple. The sensor exhibited an increase in the reduction peak current around −0.18 V by increasing [Ag+] over the range of 10–500 nM, and the peak current is saturated to 500 nM (Figure 2B). The signal of MB reduction shows a linear response to the Ag+ concentration between 10 and 200 nM with a correlation coefficient of \( R^2 = 0.95 \) and a detection limit of 10 nM, which is lower than the maximum contaminant level of Ag+ in drinking water as provided by the U.S. Environmental Protection Agency (900 nM). It is also lower than the Ag+ concentration found in the saliva of people with amalgam tooth fillings (300 nM).26 To examine the selectivity of the fabricated sensor, we compared the electrochemical signal gain in the presence of Ag+ and other metal ions such as Mg2+, Ca2+, Ba2+, Fe3+, Co2+, Ni2+, Cu2+, Zn2+, Cd2+, and Pb2+ at 500 nM (Figure 3). Among the various metal ions investigated, Ag+ exhibited an obvious signal change compared to others. Furthermore, the response of the sensor to Ag+ was not significantly affected by the presence of a mixture of other metal ions (Figure 3).
The reusability of a particular device is an important factor for practical sensor applications. We examined the reusability of the Ag⁺ sensor to determine whether the original open structure of the probe DNA can be regenerated. The strategy for the regeneration of the Ag⁺ sensor is shown in Figure 1. The sensor could be readily regenerated by the incubation of the DNA-modified electrode in 100 mM ethylenediaminetetraacetic acid (EDTA) for 30 min, which completely removed Ag⁺ through the formation of very stable C—Ag⁺—C complexes with EDTA. EDTA can bind silver(I) ions more effectively, meaning that it can remove Ag⁺ from the unfolding structure without adsorbing onto the Au electrode. The relative responses to three regenerative cycles of the same electrode are shown in Figure 4A. After the first Ag⁺ detection process, the Au electrode was incubated in 100 mM EDTA to open the hairpin structure, followed by immersion in 1 M NaClO₄ electrolyte at room temperature to reset the structure of the probe DNA. The regenerated electrode was incubated again in 100 mM EDTA for 30 min. (B) Dependence of signal gain change for each cycle of the sensor. The error bars denote the standard deviations taken from three independent experiments.

![Figure 4](https://doi.org/10.1021/acsomega.1c00410)

The dependence of sensor signaling on the probe packing density is more complex. The maximum signal gain was observed at a medium density (MD) of probe DNA on the electrode and decreased at both high density (HD) and low density (LD). The signal gain at LD exhibited little change, whereas at HD, the signal gain was approximately one-quarter of the maximum signal gain (with 100 nM Ag⁺), as shown in Table 1. This behavior appears to be caused by two competing effects: at LD, the ssDNA structure is flexible and its hairpin structure may collide with the electrode surface when binding Ag⁺ to the probe DNA, leading to no signal gain. At HD, the electrode-bound DNA becomes more densely packed, and the formation of a locked hairpin structure is limited because of the steric hindrance; consequently, the signal gain is very small. The MD probe DNA on the electrode surface exhibited optimal performance.

### 3. CONCLUSIONS

We have developed a reusable “turn-on” electrochemical silver(I) ion sensor based on selective binding with oligonucleotides. The underlying principle of the sensor is simple, but the results clearly demonstrate good sensitivity and selectivity with a detection limit of 10 nM and negligible response to other metal ions. Importantly, the system proposed in this study can be easily and rapidly regenerated without any discernible loss in activity. By controlling the probe packing density on the electrode surface, the analytical performance of the sensor can be optimized. It is expected that this method can be applied to integrated, portable, and low-cost devices for the analysis of Ag⁺ and other toxic metal ions in environmental and biological fields.

### 4. EXPERIMENTAL SECTION

#### 4.1. Materials

Thiolated MB-tagged DNA oligomer was purchased from BIONEER Corporation (Daejeon, Korea). The sequence of the modified oligomer, 5′-HS-CAA CCA ACC AAT TTT TTC CCT TC-(CH₂)₆-MB-3′, containing a thiol at 5′ and MB at 3′. Tris(hydroxymethyl)-aminomethane, silver nitrate, tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), 3-mercaptop-1-propanol (MP), sodium perchlorate, and hexammineruthenium chloride were ob-
tained from Sigma-Aldrich. EDTA and other metal nitrate salts were purchased from Daejung (Siheung, Korea). Deionized double-distilled water was used to prepare all the solutions.

4.2. Electrochemical Measurements. All electrochemical measurements were conducted using a CH Instruments (Austin, TX, USA) model 760E electrochemical analyzer in a standard three-electrode cell using gold electrodes as the working electrodes, a Pt wire as the counter electrode, and an Ag/AgCl reference electrode. All potentials were reported versus the Ag/AgCl reference at room temperature. Prior to measurements, all solutions were thoroughly purged with extra pure nitrogen gas for at least 20 min. DPV was performed at a pulse amplitude of 0.05 V and a pulse width of 0.05 s. All DPV measurements were carried out in 1 M NaClO₄ solution. CC was carried out with a pulse period of 250 ms and a pulse width of 700 mV.

4.3. Electrochemical Cleaning of the Au Electrode and Probe DNA Immobilization. A gold electrode (1.6 mm in diameter) was polished with 0.3 µm alumina (Buehler, Lake, Bluﬀ, MN) and rinsed with deionized water. Residual alumina particles were thoroughly removed by sonicating the electrodes in ethanol and deionized water for 5 min. Furthermore, it was cleaned by electrochemical oxidation and reduction in 0.5 M H₂SO₄ by applying a positive potential of +2.0 V for 5 s, followed by a negative potential of −0.35 V. Repetitive CV experiments were conducted in the potential range of −0.3 to +1.55 V at a scan rate of 4 V/s in 0.5 M H₂SO₄ until a reproducible result was achieved. The electrode was ﬁnally checked for cleanliness by running a CV cycle in a fresh 0.5 M H₂SO₄ solution at a scan rate of 0.1 V/s between −0.3 and +1.55 V (Figure 6). The surface roughness factor of the clean gold electrode was determined to be approximately 3.0. After rinsing and drying with nitrogen, the electrode was immediately used for DNA immobilization. The electrode was modiﬁed with probe DNA by incubation in a solution of thiolated MB-tagged DNA oligomers in 10 mM Tris-HCl buﬀer (pH 7.4) for 30 min at room temperature. Different

Table 1. Comparison of Sensing Performance of Electrodes with Different Probe DNA Packing Densities

| probe DNA packing            | surface density (molecules/cm²) | signal gain (%) |
|------------------------------|---------------------------------|-----------------|
| low density (0.2 µM)         | (7.2 ± 0.4) × 10¹¹             | −2.2 ± 1.6      |
| medium density (0.5 µM)      | (5.7 ± 0.9) × 10¹²             | 42.0 ± 5.4      |
| high density (2 µM)          | (9.3 ± 0.5) × 10¹²             | 10.9 ± 0.8      |

“Signal gain is deﬁned by $i_p/i_{baseline} − 1$. $i_p$ and $i_{baseline}$ are the MB reduction currents before and after the reaction with Ag⁺. The results were averaged from three independent experiments.

Figure 5. (Top) Representative chronocoulometric curves for electrodes with different probe packing densities in 10 mM Tris-HCl buﬀer (pH 7.4) in the absence (black) and presence (red) of 100 µM [Ru(NH₃)₆]³⁺. Intercept at $t = 0$ in chronocoulometric curves represent the linear fit to the data for determination. (Bottom) Differential pulse voltammograms of the sensor with different probe packing densities obtained before (black) and after (red) reaction with 100 nM Ag⁺.

Figure 6. Cyclic voltammogram acquired following electrochemical cleaning of a gold electrode in 0.5 M H₂SO₄ solution. The scan rate is 0.1 V/s. The characteristic single sharp reduction peak located around +0.9 V and multiple overlapping oxidation peaks in the range of +1.2 to 1.4 V are clearly visible.
probe packing densities were investigated in this work by controlling the concentration of the probe DNA employed during the immobilization step. Low-density (LD) surfaces were obtained by the incubation of the electrode with 0.2 μM probe DNA. MD and HD surfaces were prepared by incubating the electrodes with 0.5 and 2 μM probe DNA, respectively. Prior to immobilization, the probe DNA was mixed with 1 mM TCEP for 2 h to reduce the disulfide binding oligomer. Following the probe DNA immobilization, the electrodes were further treated with 1 mM MP for 1 h to obtain well-aligned DNA monolayers.

4.4. Determination of the Surface Coverage. The density of surface-immobilized DNAs can be obtained by the assumption that redox-active \([\text{Ru(NH}_3\text{)}_6\text{]}^{3+}\) molecule stoichiometrically binds to the anionic phosphate backbone of DNA. \(^{32}\) The surface coverage of the probe DNA can then be calculated from the redox charges of \([\text{Ru(NH}_3\text{)}_6\text{]}^{3+}\) according to chronocoulometric methods, first proposed by Tarlov and co-workers. \(^{33}\) It can be calculated from the following equations:

\[
\Gamma_{\text{DNA}} = \Gamma_0 (z/m) N_A \quad (1)
\]

\[
\Gamma_0 = Q/(nFA) \quad (2)
\]

where \(\Gamma_{\text{DNA}}\) is the surface density of DNA (mol/cm²), \(\Gamma_0\) is the surface density of \([\text{Ru(NH}_3\text{)}_6\text{]}^{3+}\) (mol/cm²), \(z\) is the charge of the redox molecule, \(m\) is the number of nucleotides in the DNA, \(n\) is the number of electrons in the reaction, \(A\) is the area of the working electrode (cm²), \(F\) is the Faraday constant (C/mol), \(N_A\) is Avogadro’s number, and \(Q\) is the charge that can be obtained from calculating the chronocoulometric intercept at \(t = 0\). CC provided an accurate measure of the surface excess of redox markers at a DNA-modified electrode, which was then employed in the present experiments. \(^{35}\)

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**Notes**

The authors declare no competing financial interest.

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