Colloids and Surfaces B: Biointerfaces 167 (2018) 150–155

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

# DNA-functionalized gold nanoparticle-based fluorescence polarization for the sensitive detection of silver ions

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ARTICLE INFO

*Article history:*
Received 12 January 2018
Received in revised form 29 March 2018
Accepted 2 April 2018
Available online 5 April 2018

*Keywords:*
Gold nanoparticle
Fluorescence polarization
Biosensor
$Ag^+$ detection

ABSTRACT

Despite their practical applications, $Ag^+$ ions are environmental pollutants and affect human health. So the effective detection methods of $Ag^+$ ions are imperative. Herein, we developed a simple, sensitive, selective, and cost-effective fluorescence polarization sensor for $Ag^+$ detection in aqueous solution using thiol-DNA-functionalized gold nanoparticles (AuNPs). In this sensing strategy, $Ag^+$ ions can specifically interact with a cytosine–cytosine (C—C) mismatch in DNA duplexes and form stable metal-mediated cytosine–$Ag^+$–cytosine (C–$Ag^+$–C) base pairs. The formation of the C–$Ag^+$–C complex results in evident changes in the molecular volume and fluorescence polarization signal. To achieve our aims, we prepared two complementary DNA strands containing C-base mismatches (probe A: 5′-SH-$A_{10}$-TACCACTCCTCAC-3′ and probe B: 5′-TCCTCACCAGTCCTA-FAM-3′). The stable hybridization between probe A and probe B occurs with the formation of the C–$Ag^+$–C complex in the presence of $Ag^+$ ions, leading to obvious fluorescence quenching in comparison to the system without AuNP enhancement. The assay can be used to identify nanomolar levels of $Ag^+$ within 6 min at room temperature, and has extremely high specificity for $Ag^+$, even in the presence of higher concentrations of interfering metal ions. Furthermore, the sensor was successfully applied to the detection of $Ag^+$ ions in environmental water samples and showed excellent selectivity and high sensitivity, implying its promising application in the future.



## 1. Introduction

$Ag^+$ ions have excellent antibiotic and bactericidal effects. They also have wide uses in the fields of photography, video, electronics, pharmaceuticals, biosensing, etc. However, $Ag^+$ ions are considered hazardous metal ions and are a major environmental pollutant. Furthermore, they can destroy the activity of enzymes and combine with amine, imidazole, and various metabolites, with negative consequences for environmental safety and human health [1–7]. Owing to these adverse effects, the development of a rapid and simple method for $Ag^+$ detection is imperative. Conventional methods, such as flame atomic absorption, potentiometry, voltammetry, inductively coupled plasma atomic emission spectrometry, and fluorescence methods, are widely used to detect $Ag^+$ ions. However, these methods have various shortcomings, e.g., they are generally time-consuming, expensive, and complicated [8–12]. Therefore, it is imperative to develop a sensitive and selective biosensor for $Ag^+$ ion detection [13–16].

In the last few decades, extensive efforts have focused on the design of sensing systems for the detection of $Ag^+$ ions, including sensors based on fluorescent chromophores [17], semiconductor quantum dots [18,19], colorimetric sensors [20,21], and fluorescent sensors [22,23]. However, many of these systems have major limitations, such as interference from closely related metal ions, high costs, and complicated analytical procedures. Recently, the high specificity of the interaction of oligonucleotides with metal ions has been successfully and widely used for studies of heavy metal ion detection. Since Ono et al. first found that $Ag^+$ can selectively coordinate cytosine (C) bases to form a stable C–$Ag^+$–C complex, an assay based on the specific coordination of $Ag^+$ with C bases has been widely employed for monitoring $Ag^+$ ions [24]. Zhao et al. reported a reusable DNA single-walled carbon nanotube-based fluorescent sensor for the highly sensitive and selective detection of $Ag^+$ and cysteine (Cys) in aqueous solution. This approach enabled the simultaneous detection of two objects, but the detection range was relatively narrow [25]. Lv et al. described a reversible fluores-

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https://doi.org/10.1016/j.colsurfb.2018.04.004
0927-7765/

cent DNA–based INHIBIT logic gate for the determination of $Ag^+$ and $I^-$. In this method, graphene oxide was used as a signal transducer and Ag(I) and iodide as mechanical activators. Although this biosensor was highly sensitive with low background interference, highly toxic AgI was introduced in this system [26].

Sensors based on gold nanoparticles (AuNPs) are promising for the simple and rapid tracking of $Ag^+$ based on the extreme sensitivity of the surface plasmon resonance absorbance of AuNPs [27–29]. For example, Lin et al. reported a colorimetric method for the detection of $Hg^{2+}$ and $Ag^+$ using Tween 20-modified AuNPs [30]. Li et al. developed both unlabeled and labeled sensing strategies for $Ag^+$ ions using the DNA-based AuNPs colorimetric strategy [31]. However, these two approaches are susceptible to severe interference from other metal ions and typically require masking agents. These masking agents are often highly toxic, resulting in additional environmental contamination and complicated analytical procedures. Likewise, an on–off sensor based on DNA-functionalized AuNPs for silver ions and cysteine detection using a light-scattering technique has been generated, but a satisfactory limit of detection (LOD) has not been obtained [32]. Zhou et al. designed a novel biosensor based on nanoporous gold and duplex-like DNA scaffolds with an anionic intercalator to realize $Ag^+$ ion detection [33]. Additionally, an AuNP and enzyme cleavage-based dual signal amplification strategy for ultrasensitive detection of $Ag^+$ using electrochemical techniques has been proposed by Miao et al. [34]. These sensing platforms for $Ag^+$ detection have extremely low LODs and high selectivity over other environmentally relevant metal ions, but require expensive instruments and complex procedures. Thus, the development of a highly sensitive, simple, rapid, and cost-effective assay for $Ag^+$ ion detection remains a challenge.

Inspired by previous reports, we proposed a simple, rapid, and highly selective sensor for the detection of $Ag^+$ using cytosine–$Ag^+$–cytosine (C–$Ag^+$–C) coordination chemistry and a fluorescence polarization technique enhanced by AuNPs and optimized the experimental conditions. This novel sensor is very simple and cost-effective, and can identify $Ag^+$ at the nanomolar level (LOD = 9.5 nM) using widely available materials and instruments at room temperature. In addition, the analysis procedure is very rapid, which only takes approximately 6 min to achieve $Ag^+$ detection. Moreover, in the presence of other competitive metal ions, this biosensor is highly selective for $Ag^+$ without masking agents, minimizing additional environmental pollution. Most important, the proposed strategy may have broad applications for the monitoring of other metal ions by applying different DNA strands of specific sequences.

## 2. Materials and methods

### 2.1. Materials and instruments

All chemicals used in this work were obtained from commercial sources and used directly, without further purification. Trisodium citrate and hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4{\cdot}3H_2O$, purity ≥ 99.9%) were purchased from Sigma (St. Louis, MO, USA). Probe A (5′-SH-$A_{10}$-TACCACTCCTCAC-3′) and probe B (5′-TCCTCACCAGTCCTA-FAM-3′) were synthesized using a standard procedure and purified by reverse-phase high-performance liquid chromatography by Sangon Inc. (Shanghai, China). Silver nitrate ($AgNO_3$, purity ≥ 99.5%), sodium nitrate ($NaNO_3$, purity ≥ 99.9%), and tris-(2-carboxyethyl) phosphine hydrochloride (TCEP, purity ≥ 99%) were purchased from Alfa Aesar (Haverhill, MA, USA). $AgNO_3$ was weighed to prepare a stock solution, which was diluted to the desired concentrations for the experiments. The concentration of the $AgNO_3$ solution was calibrated by atomic absorption spectrophotometry. Other metal ions were of analytical grade and were purchased from Shanghai Sinopharm (Shanghai, China). Water used in all experiments was double-distilled.

Fluorescence polarization was performed using a Cary Eclipse Fluorescence Spectrophotometer equipped with a normal polaroid (Varian, Palo Alto, CA, USA). UV–vis spectra were recorded on a TU-1810 spectrophotometer (Puxi Analytic Instrument Ltd., Shanghai, China). Transmission electron microscopy (TEM) images were obtained using a JEM 2100 (JEOL, Tokyo, Japan) operated at 200 kV. Flame atomic absorption spectroscopy results were recorded using the aZ-5000 Flame Atomic Absorption Spectrophotometer (Hitachi, Tokyo, Japan). Centrifugation was performed using a high-speed refrigerated centrifuge (Shanghai Anting Scientific Instrument Co., Ltd., Shanghai, China). All pH measurements were obtained using a Model pHS-2C pH meter (Shanghai Dapu Instrument Co., Ltd., Shanghai, China). Temperature was maintained in a low-temperature thermostat bath (Shanghai Ping Xuan Science Instrument Co., Ltd., Shanghai, China).

### 2.2. Synthesis and functionalization of AuNPs

Citrate-capped AuNPs were prepared by the chemical reduction of $HAuCl_4{\cdot}3H_2O$ in the liquid phase [6]. All glassware was cleaned in aqua regia and rinsed with double-distilled water prior to use. A 0.01% $HAuCl_4$ solution (100 mL) was brought to a vigorous boil with stirring in a round-bottom flask fitted with a reflux condenser, and 3.5 mL of 1% trisodium citrate solution was rapidly added to the boiling solution. Finally, the mixture was heated under reflux for another 15 min. During this time, the mixture changed from pale yellow to deep red. The mixture was cooled to room temperature, while stirring continuously. Then, it was filtered through a 0.45-μm membrane filter and stored in a refrigerator at 4 °C. AuNPs were nearly monodispersed, and the average size was 13 ± 2 nm based on a TEM analysis. The concentration of AuNPs was evaluated by UV–vis spectroscopy using an extinction coefficient of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at λ = 520 nm (Fig. 1) [35].

Before the functionalization of AuNPs, probe oligonucleotides were deprotected by soaking in 20 mM Tris-$CH_3COOH$ buffer solution (pH 7.0) containing 100 mM TCEP for at least 2 h. AuNPs were functionalized with the probe A following the normal procedure. The solution was incubated at 25 °C for 12 h in 10 mM Tris-$CH_3COOH$ (pH 7.0). Then, the NaCl solution was gradually added, and the final concentration of NaCl was 0.1 M. Finally, the solution was allowed to "age" under these conditions for an additional 24 h to form DNA–AuNPs complexes. Excess reagents were removed by centrifugation at 21,560 × *g* (14,000 rpm) and 4 °C for at least 20 min. Following removal of the supernatants, the oily precipitates were washed with 10 mM Tris-$CH_3COOH$ (pH 7.0). After three centrifuge/wash cycles, the colloidal solution was suspended in stock solution and placed in a refrigerator until further use. Before each experiment, the UV–vis absorbance of the stock solution of DNA–AuNPs was determined, and the constant absorption spectrum confirmed the stability of DNA-AuNPs.

### 2.3. Effect of gold nanoparticles on probe B

AuNPs (50 nM) were added to the probe B solution, and the fluorescence polarization values of the mixed solution and single probe B solution were measured at an excitation wavelength of 485 nm. The effect of AuNPs on the fluorescence polarization value of the probe B solution was evaluated.

### 2.4. Fluorescence polarization measurements

The prepared AuNP-DNA and probe B solutions were mixed with equal volumes, and then the $AgNO_3$ standard solution was added to