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i-Motif-modulated fluorescence detection of silver(I) with an ultrahigh specificity

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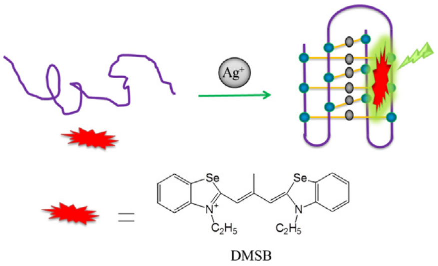
HIGHLIGHTS

- Ag^+ shows a high specificity for inducing i-motif formation.
- A cyanine dye was designed to recognize i-motif formation.
- A novel Ag^+ sensor is designed.
- The sensor shows excellent performance in specificity, sensitivity, and real time response.
- Detection of the Ag^+ level in freshwater system is proved.

GRAPHICAL ABSTRACT

The scheme for sensing Ag^+ based on DMSB recognizing motif formation and the molecular structure of DMSB.



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ABSTRACT

A novel Ag^+ sensor has been designed based on the mechanism that i-motif formation induced by Ag^+ was sensitively recognized by a cyanine dye. The sensor exhibited an over 130–16,000 fold selectivity toward Ag^+ than that toward other metal ions. This research not only provides a step forward toward the development of Ag^+ detection but also represents a new application for i-motif DNA.

1. Introduction

Silver ion (Ag^+) has been considered to be a high-effect fungicide for a long time. It is always thought to be a precious metal safe to humans. Therefore, silver ions and its containing compounds are widely used in biomedical, jewelry, and cloud seeding field. It also has widespread applications in the electrical and electronic industry, imaging industry, and the manufacturing of fungicides. However, a massive quantity of Ag^+ is released to environment annually from industrial wastes and emissions. Excessive amounts of Ag^+ are distributed and contaminated in ambient air, water, and soil, which may lead to serious environmental problems. Ag^+ can accumulate in human body through the food chain, and causes toxic side effects to the human health. For example, silver ions can bind with varies metabolites and enzymes, causing various diseases and loss of protein function. Therefore, there is an urgent demand for simple and highly specific methods for the analysis of silver ions.

To date, various techniques such as atomic absorption or emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-

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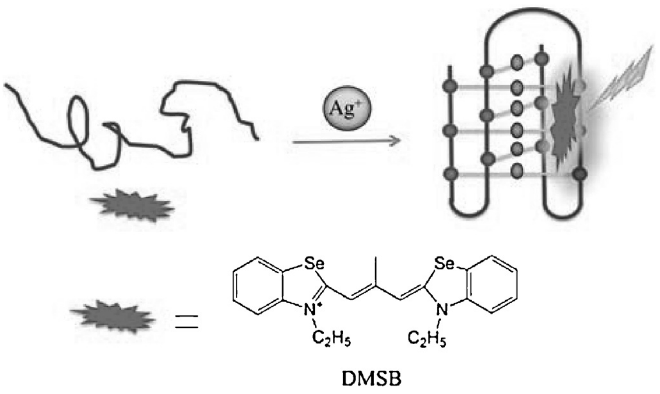

Scheme 1. The scheme for sensing Ag$^+$ based on DMSB recognizing motif formation and the molecular structure of DMSB.

MS), electrochemical methods, ion exchange chromatography (IEC), and laser excited atomic fluorescence spectrometry have been applied to the determination of Ag$^+$ in aqueous samples. However, these traditional methods require sophisticated and high cost instruments, and complicated sample preparation, which might limit their application in the routine monitoring of silver ions. Recently, the design of molecular sensors of Ag$^+$ has attracted immense interest [1–9], and some of them have been developed based on the binding of Ag$^+$ to two cytosine (C) residues of DNA to form the C–Ag$^+$–C complex [10–17]. Nevertheless, these methods require elaborate sample preparation and precise techniques, and their selectivity to Ag$^+$ also need to improve. Consequently, detection of Ag$^+$ using a simple method with high selectivity still remains a great challenge.

In recent years, i-motif has attracted considerable attention due to its potential application in nanotechnology and physiological roles in gene transcription. i-Motif DNA is a type of four-stranded nucleic acid secondary structure, which is comprised of two parallel duplexes hydrogen bonded together in an antiparallel orientation by intercalated C$^+$–C base pairs [18–21]. To promote i-motif formation, an acidic condition is generally needed, which is proposed to protonate the N3 in cytosine. However, recently Waller et al. reported that Ag$^+$ is able to specifically interact with the C–C mismatch in DNA duplexes to form C–Ag$^+$–C complex, which can enable single-stranded DNA to form folded i-motif structure rather than a hairpin duplex structure at neutral pH and room temperature [22]. Based on the property of Ag$^+$ inducing DNA to form i-motif structure, selective detection of Ag$^+$ is possible by recognizing the formation of DNA i-motif.

Inspired by this idea, we designed a new Ag$^+$ sensor based on recognizing i-motif formation. A cyanine dye 2,2-diethyl-9-methyl-selenacarbocyanine bromide (DMSB, Scheme 1) is selected to recognizing i-motif. With i-motif being induced by Ag$^+$, the fluorescence intensity of DMSB is obviously enhanced, which eventually enables us to detect Ag$^+$ by monitoring the fluorescence intensity of DMSB. To the best of our knowledge, it is the first Ag$^+$ sensor based on a ligand recognizing i-motif structure. This probe exhibits excellent selectivity and shows great promise for real sample analysis, representing a step forward toward the development of silver(I) ion detection.

2. Materials and methods

2.1. Materials

The oligonucleotides cAS1411 (5′-CCACCACCACCACAACCACCACCACCAAA-3′), TBA (5′-CCAACCACACCAACCAAA-3′), C-Kit2 (5′-C$_3$TC$_3$TCGCGC$_3$GC$_3$GA$_3$-3′), HIF-1α (5′-C$_3$GC$_5$TCTC$_3$TC$_3$A$_3$-3′), VEGF (5′-C$_3$GC$_5$GGC$_3$GC$_3$A$_3$-3′), Telomeric (5′-C$_3$TAAC$_3$TAAC$_3$TAAC$_3$T-3′), Bcl-2 (5′-C$_3$GC$_3$AATTCCTC$_3$GCGC$_3$-3′), and C-myc (5′-CCTTC$_4$AC$_3$TC$_4$AC$_3$TC$_4$A-3′) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China), purified by PAGE. NaH$_2$PO$_4$, Na$_2$HPO$_4$ and ethylenediaminetetraacetic acid (EDTA) of analytical quality were purchased from Sigma–Aldrich (Saint-Quentin, France). The cyanine dye DMSB was synthesized according to Hamer's [23] and Brooker's [24] methods.

2.2. Sample preparation

The stock solution of DMSB was prepared by dissolving it in methanol to 200 μM and then stored in the dark at −4 °C. The stock solutions of the oligonucleotides were prepared by dissolving oligonucleotides directly into 10 mM PB buffer solution (10 mM NaH$_2$PO$_4$/Na$_2$HPO$_4$, 1 mM EDTA, pH 7.2). The concentrations of DNA stock solutions were determined by measuring their absorbance at 260 nm. All DNA samples were structurally identified by circular dichroism (CD) spectra. Ultrapure water prepared by Milli-Q Gradient ultrapure water system (Millipore) was used throughout the experiments.

2.3. Fluorescence measurements

Fluorescence spectra were taken on a Hitachi F-4500 spectrophotometer in a 10 mm quartz cell at room temperature. Xenon arc lamp was used in the excitation light source in fluorescence measurement. The excitation wavelength was 520 nm. Both excitation and emission slits were 5 nm and the voltage was 700 V with a scan speed of 1200 nm min^{-1}.

2.4. CD measurements

CD spectra were collected from 200 to 350 nm on a Jasco-815 automatic recording spectropolarimeter with a 1-cm path-length quartz cell at 25 °C. Spectra were collected with scan speed of 500 nm min^{-1}. Each spectrum was the average of three scans. A solution containing no oligonucleotide was used as reference, and a buffer blank correction was made for all spectra. The temperature of the cell holder was regulated by a JASCO PTC-423S temperature controller. The cuvette-holding chamber was flushed with a constant stream of dry N$_2$ gas to avoid water condensation on the cuvette exterior.

3. Results and discussion

3.1. The specific response of i-motif toward Ag$^+$

The response of C-rich oligonucleotides to Ag$^+$ was determined by measuring the CD spectra. A red-shift effect of the CD positive peak was observed with increasing Ag$^+$ concentrations (Fig. S1), corresponding to i-motif formation [25]. As a comparison, other metal ions were also used to interact with the oligonucleotides under the same conditions as that of Ag$^+$. However, the i-motif structure only formed when Ag$^+$ was present (Fig. S2), meaning Ag$^+$ has an extremely high specificity versus other cations for inducing i-motif. This result implicates that i-motif will show an excellent performance in developing the Ag$^+$ sensor.

3.2. DMSB as a reporter of i-motif formation

To monitor Ag$^+$ by recognizing i-motif, a probe that can report i-motif formation is required. In previous studies, targeting G-quadruplex, another four-stranded DNA structure, we developed a series of probes with high specificity and sensitivity [26–29]. These