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A test strip platform based on DNA-functionalized gold nanoparticles for on-site detection of mercury (II) ions

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ABSTRACT

A test strip, based on DNA-functionalized gold nanoparticles for Hg^{2+} detection, has been developed, optimized and validated. The developed colorimetric mercury sensor system exhibited a highly sensitive and selective response to mercury. The measurement principle is based on thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) coordination chemistry and streptavidin–biotin interaction. A biotin-labeled and thiolated DNA was immobilized on the gold nanoparticles (AuNPs) surface through a self-assembling method. Another thymine-rich DNA, which was introduced to form DNA duplexes on the AuNPs surface with thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) coordination in the presence of Hg^{2+}, was immobilized on the nitrocellulose membrane as the test zone. When Hg^{2+} ions were introduced into this system, they induced the two strands of DNA to intertwist by forming T—Hg^{2+}—T bonds resulting in a red line at the test zone. The biotin-labeled and thiolated DNA-functionalized AuNPs could be captured by streptavidin which was immobilized on the nitrocellulose membrane as the control zone. Under optimized conditions, the detection limit for Hg^{2+} was 3 nM, which is lower than the 10 nM, maximum contaminant limit defined by the US Environmental Protection Agency (EPA) for drinking water. A parallel analysis of Hg^{2+} in pool water samples using cold vapor atomic absorption spectrometry showed comparable results to those obtained from the strip test. Therefore, the results obtained in this study could be used as basic research for the development of Hg^{2+} detection, and the method developed could be a potential on-site screening tool for the rapid detection of Hg^{2+} in different water samples without special instrumentation. All experimental variables that influence the test strip response were optimized and reported.

1. Introduction

Mercury ion (Hg^{2+}) is one of the most toxic metallic pollutants that exerts harmful biological effects on the environmental and human health. Exposure to even very low levels of mercury can cause health damage to human. Mercury is accumulated in the vital organs and tissues, where it binds with sulfur-containing proteins and enzymes, causing organ dysfunction and a devastating effect on the whole central nervous system of human [1–3]. Mercury pollution received considerable public attention after the mercury poisoning incident referred as Minamata disease in Japan during 1950s. To protect public health, many countries and organizations have established the limits for Hg^{2+} in water samples. For example, the US Environmental Protection Agency (EPA) has set a maximum limit of 10 nM Hg^{2+} in drinking water [4]; whereas, the World Health Organization (WHO) permits a maximum level of 30 nM Hg^{2+} in drinking water [5]. With increasing pollution in the environmental waters, there is an urgent need for the rapid detection of Hg^{2+} in water samples.

Many analytical techniques are available to determine Hg^{2+}, for example, chromatography [6], inductively coupled plasma-mass spectrometry (ICP-MS) [7], atomic absorption/emission spectrometry [8,9], cold vapor atomic absorption spectrometry (CVAAS) [10,11], cold vapor atomic fluorescence spectrometry [12], electrochemiluminescence [13,14] and other electrochemical methods [15]. Despite the high sensitivity and specificity that can be achieved by these methods for the determination of Hg^{2+}, they require expensive instrumentation, time-consuming sample pretreatment and highly skilled personnel, and/or have cross-sensitivities toward other metal ions. Thus, a simple, direct and inexpensive method for detecting Hg^{2+} remains desirable.

One potential approach is the colorimetric method based on gold nanoparticles (AuNPs), which is extremely attractive, because it can be easily read out with the naked eye, in some cases at the point of use. DNA-functionalized AuNPs have become interesting nanomaterials for sensing Hg^{2+}, mainly because AuNPs are good indicators of binding and hybridization events, and Hg^{2+} can bind between thymines to form stable thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) base pairs in DNA [16,17]. In recent years, a variety of

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colorimetric methods for detecting Hg^{2+} in aqueous media using DNA-functionalized nanoparticles have been reported [18–22]. However, two disadvantages have prevented their application for the on-site detection of Hg^{2+}. First, detection still requires professional instrumentation and highly skilled personnel, thus making it less useful for people who do not have a scientific background. Second, in aqueous media, the AuNPs give a strong red background. Although the sensitivity is high when the absorbance is recorded using a UV–vis spectrophotometer, it is often difficult to distinguish the blue color of aggregates against the red background from the dispersed nanoparticles by visual observation. At low Hg^{2+} concentrations, only a small fraction of nanoparticles aggregate and change to a blue color, which is likely to be masked by the strong red background.

The test strip platform based on DNA-functionalized AuNPs is well suited for one-step detection of Hg^{2+}, which has been used for DNA analysis [23], genotyping [24,25], protein analysis [26] and small molecule detection [27,28]. Torabi and Lu [29] recently reported a colorimetric sensor with functional DNA-linked AuNPs to achieve a detection limit of 5.4 nM for Hg^{2+} by determining the color change of the sensor on the dipstick test. Although this system has a high sensitivity for determination of Hg^{2+}, it has a disadvantage that AuNPs are vulnerable to aggregation, thus it is difficult to make AuNPs dissociate from the aggregates and change to a red color. Thereby, it can easily give false negative results. He et al. [30] also developed a colorimetric strip based on AuNPs and thymine-rich hairpin DNA probes. Nevertheless, the assay required three different sequences of DNA modified in three different groups for the detection. Instead, Zhou et al. [31] reported a competitive immunochromatographic assay for the detection of Hg^{2+}. In the absence of Hg^{2+}, two visible lines would appear; whereas, in the presence of Hg^{2+}, the density of the test line would be lighter than that of the control line. When the concentration of Hg^{2+} is high enough, the test line would disappear. This detection principle has a disadvantage that it is difficult to visually distinguish the slight differences between two lines when the concentration of Hg^{2+} approached the detection limit.

In this work, we designed a colorimetric mercury sensor system for the detection of Hg^{2+} using DNA-functionalized AuNPs. Based on the sensor system, a simple, highly selective and sensitive test strip for the on-site and colorimetric detection of Hg^{2+} at nM level was thus developed. The sensitivity achieved by this method is the highest among the reported methodologies. The mechanism of detection was also discussed.

2. Materials and methods

2.1. Chemicals and materials

Streptavidin from *Streptomyces avidinii*, bovine serum albumin (BSA) and guanidine hydrochloride (Gu-HCl) were purchased from Sigma–Aldrich (Steinhem, Germany). Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) was purchased from Pierce (Interchim, Montluçon, France). Two kinds of thymine-rich single-stranded DNA with different sequences, i.e. DNA_1 and DNA_2, as shown in Fig. 1, were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4{\cdot}4H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical reagent grade and were used without further purification or treatment.

The AuNPs with a mean diameter of 13 nm were synthesized as described previously [32,33]. All metal ion solutions were prepared from nitrate salts. Working solutions of DNA_1, DNA_2 and streptavidin were prepared with 20 mM Tris buffer solution (pH 7.2), which contained 100 mM $NaNO_3$ and 8% sucrose. Milli-Q water (18 MΩ cm) was used throughout the experiment.

Hi-Flow Plus Assembly Kit was purchased from Millipore Corporation (Bedford, MA, USA), containing a Hi-Flow nitrocellulose (NC) membrane, cellulose fiber, glass fiber and plastic adhesive packing.

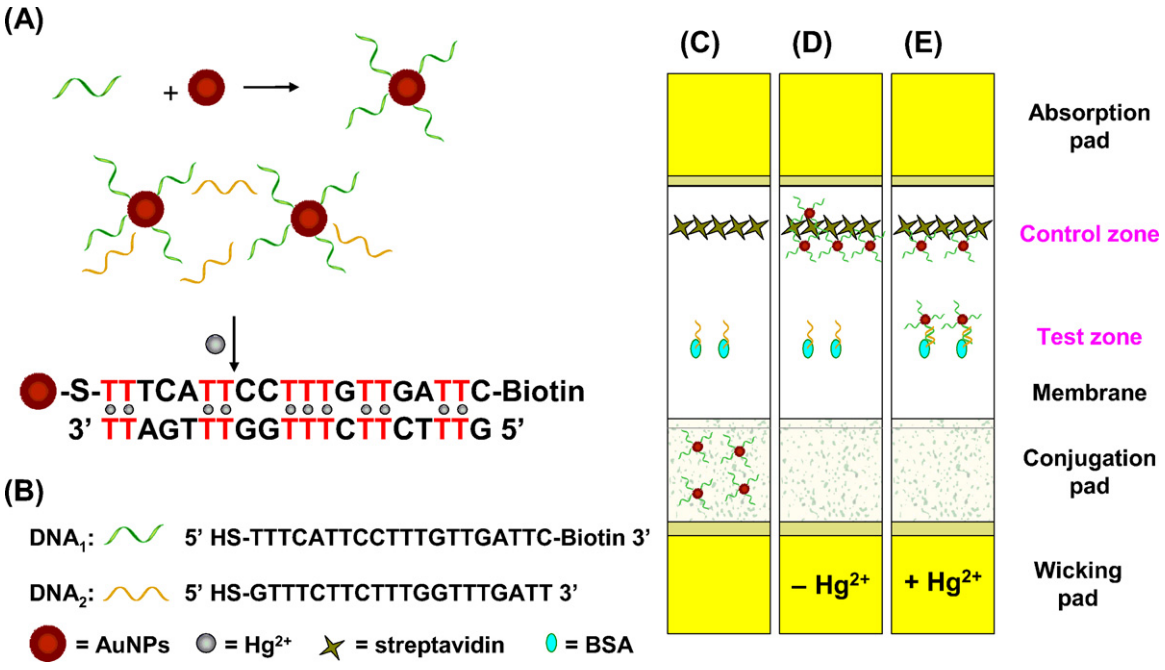

Fig. 1. Design of the test strip format. (A) Schematic illustration of sensors and theranostic agents for Hg^{2+}. (B) Description of DNA_1 sequence, DNA_2 sequence, AuNPs, Hg^{2+}, streptavidin and BSA. (C) Blank test strip loaded with the DNA-functionalized AuNPs (on the conjugation pad), DNA_2–BSA (test zone) and streptavidin (control zone). (D) Negative test: in the absence of Hg^{2+}, the DNA-functionalized AuNPs were captured at the control zone through streptavidin–biotin interaction, producing a single red line. (E) Positive test: in the presence of Hg^{2+}, the DNA-functionalized AuNPs were captured at the test zone by T—Hg^{2+}—T coordination to complementary DNA in addition to the control zone resulting in two red lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)