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Highly sensitive fluorescence detection of mercury (II) ions based on WS_2 nanosheets and T7 exonuclease assisted cyclic enzymatic amplification

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ABSTRACT

In this work, a simple and signal-on assay has been developed for highly sensitive and selective detection of mercury ion (Hg^{2+}) based on the WS_2 nanosheets and T7 exonuclease-assisted cyclic signal amplification. WS_2 nanosheet exhibits differential affinity toward short oligonucleotide fragment versus single-stranded DNA (ssDNA) probe, which is used as an adsorption material for capturing ssDNA and an efficient fluorescence quencher for reducing the background signal. T7 exonuclease, a sequence independent nuclease, catalyzes the removal of 5′ mononucleotides from the 5′ termini of double stranded DNA, while its activity on ssDNA is limited. Without Hg^{2+}, FAM-labelled ssDNA probe and target probe are adsorbed by the WS_2 nanosheet and the fluorescence of FAM-labelled signal probe is quenched. In the presence of Hg^{2+}, a FAM-labelled ssDNA probe could hybridize with the target probe to form duplex structures with a blunt 5′-terminal of signal probe through the formation of T-Hg^{2+}-T base pairing. The FAM-labelled signal probe with a blunt 5′-terminal in the formed duplex can be digested by T7 exonuclease in the direction from 5′ to 3′, liberating the FAM fluorophore and releasing the Hg^{2+}. The released target Hg^{2+} and the remaining probe then bind another FAM-labelled ssDNA, and initiate the next round of cleavage, resulting in the release of numerous FAM labels back into the solution and significantly amplified fluorescent signal. This approach can warrant the detection limit for Hg^{2+} down to 0.1 nM (S/N = 3) with high selectivity against other metal ions. Moreover, the application of the sensor for lake water shows that the proposed method works well for real samples. This research demonstrates an alternative approach to detect targets of interest that holds high prospects for detecting other biomolecules or metal ions in the near future.

1. Introduction

Environmental pollution by heavy metal ions is wide spread and the resulting adverse effects on the environment and human health have generated extensive concern [1]. Mercury ion (Hg^{2+}) is well known as an important environmental pollutant, it is the most toxic metal contaminant, even at low concentrations, that exists widely in our environment. Mercury and mercury compounds invade the human body through the respiratory and digestive tracts and the skin, and can causes serious human health problems, such as DNA damage, brain damage, organic functions disablement, and immune system homeostasis disruption [2–4]. Hence, effective methods featured with simplicity, high sensitivity and selectivity are urgently needed for Hg^{2+} detection.

Traditional analytical techniques including atomic absorption spectroscopy, atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry, and X-ray absorption spectroscopy have been widely used for measuring Hg^{2+} [5,6]. These methods are time-consuming and require complicated instruments. Due to the intrinsically high sensitivity, simplicity, and ease of operation, fluorescence-based strategies have received intensive attention. For instance, organic small-molecule fluorescence probes [7], conjugated polymers [8], polymeric materials [9], carbon nanotube [10], and gold nanoparticles-based fluorescent sensors were reported [11]. These probes take advantage of their optical property changes upon binding to Hg^{2+}, but most of them

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still suffer from disadvantages such as poor solubility in aqueous media and sophisticated synthesis of the probe materials. Therefore, it is highly crucial to develop simple, sensitive, and selective techniques for the routine monitoring of Hg^{2+} in environmental samples.

With the tremendous progress of nanotechnology, a wide diversity of nanomaterials have been prepared with reliable synthetic methods and their compelling applications in various practical areas have been demonstrated [12–16]. Graphene, a two-dimensional nanomaterial, has been extensively used in biological applications [17–20]. In recent years, transition metal dichalcogenides (e.g., MoS_2, WS_2, etc.) as two-dimensional layered nanomaterials analogous to graphene have received much attention owing to their unusual structures with high specific surface area and remarkable electronic properties catering for intriguing applications in optoelectronics and sensors [21–23]. WS_2 nanosheets are archetypical examples of inorganic analogues of graphene, the layer of a WS_2 is three atoms thick with the composition of hexagonal layer of metal atoms W sandwiched between two layers of chalcogen atoms [24]. Compared with graphene, WS_2 nanosheets can be synthesized in large scale, and can be directly dispersed in water-soluble solution, implying that WS_2 nanosheets holds great promise as a novel nanomaterial for biomedical applications [25]. It has also been demonstrated that WS_2 nanosheet exhibits a capability for discriminating single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), which may catalyze the applications of transition metal dichalcogenides nanosheets in biomedical areas [26]. In our early research, WS_2 nanosheets can also act as an efficient quencher for fluorescence dyes such as fluorescein [27]. To the best of our knowledge, the exploration of WS_2 nanosheet in biochemical application still remains at a very early stage. Moreover, it is still significant to acquire layered WS_2 nanosheet and explore its application as a universal biosensing platform.

Herein, we report a new method for the highly sensitive and selective detection of Hg^{2+} base on the WS_2 nanosheets and T7 exonuclease-assisted cyclic signal amplification. As the intrinsic and specific interaction between Hg^{2+} and thymine (T), Hg^{2+} can interact specially with thymine (T) to form stable T-Hg^{2+}-T structure [28]. T7 exonuclease, a sequence independent nuclease, catalyzes the removal of 5′ mononucleotides from the 5′ termini of double stranded DNA, while its activity on single-stranded DNA is limited [29,30]. As illustrated in Scheme 1, this strategy relies on a target recycling amplification through the specific T7 exonuclease catalyzed digestion of FAM-labelled ssDNA probes (Probe 2), which hybridize with the target ssDNA sequences (Probe 1) via the T-Hg^{2+}-T mismatch in presence of target Hg^{2+}. In our design, Probe 1 contains a long single-stranded region (24 bases) for binding WS_2 and a short double-stranded region (12 bases) for hybridize with the Probe 2 via the T-Hg^{2+}-T mismatch in presence of target Hg^{2+}. Here, because of the strong interaction between the long single-stranded tail and WS_2, the short double-stranded DNA region is able to be tightly tethered on the surface of WS_2, which makes the fluorophore close to the surface of WS_2 accompanying with an efficient FRET. The formed duplex will become the substrate for T7 exonuclease, since T7 exonuclease only catalyzes the removal of 5′ mononucleotides from the 5′ termini of dsDNA, and produces short oligonucleotide fragments and releases the Hg^{2+}. The cleaved short FAM-linked oligonucleotide fragments will not be adsorbed on the WS_2 nanosheets because of their weak affinity and thus retain a strong fluorescence signal. At the same time, the released target Hg^{2+} and the remaining probe then bind another FAM-labelled ssDNA, and initiate the next round of cleavage, resulting in significant fluorescence signal amplification. The signal amplification was achieved by the recycling of Hg^{2+} and Probe 1. In contrast, without Hg^{2+}, Probe 2 will not be digested by T7 exonuclease, their fluorescence is almost entirely quenched due to their strong affinity to WS_2 nanosheets. Therefore, by taking full advantage of the unique features of T7 exonuclease-assisted cyclic signal amplification and the super fluorescence quenching efficiency of WS_2 nanosheets, the proposed strategy provides a convenient, sensitive and selective fluorescence assay of the Hg^{2+}.

2. Experimental

2.1. Materials and apparatus

The Probe 1 and Probe 2 were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). The sequences of the DNA oligonucleotides were as follows:

Probe 1: 5′ – TCTAAAGAATAACAGTGATAATTTCTGGGTTAAG-GTAATAG-3′

Probe 2: 5′ –FAM-TCCTTAACCCAG-3′

T7 exonuclease was purchased from New England Biolabs (NEB, U.K.). Bulk tungsten sulfide (WS_2) was commercially available from Aladdin (Shanghai, China). Polyacrylic acid (PAA, with an average molecular weight of 1800) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The used metal salts ($Hg(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Cd(NO_3)_2$, $AgNO_3$, $MgCl_2$, $CaCl_2$, $FeCl_3$, $ZnCl_2$, $CuCl_2$, and $MnCl_2$) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were used as received without further purification. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.2 MΩ.

The fluorescence measurements were carried out on an FL-4600 spectrophotometer (Hitachi, Japan). The fluorescence emission spectra were collected from 500 nm to 600 nm at room temperature with a 488 nm excitation wavelength.

2.2. Synthesis of layered WS_2 nanosheet

WS_2 nanosheets were prepared according to Liu method [25]. The PAA modified WS_2 nanosheet was synthesized from bulk WS_2 by sonication-assisted liquid exfoliation in water. In a typical procedure, WS_2 powder (150 mg) and PAA (50 mg) were added to a 50 mL flask containing 20 mL of ultrapure water as the solvent and the mixture was sonicated for 6 h. Then, the dark green dispersion was centrifugated at 3000 rpm for 10 min to remove large-size masses. The supernatant was collected and further washed with water by centrifugation at 10000 rpm for 5 min. The as-obtained black precipitate was dispersed in water for further characterizations and applications.

2.3. Characterizations

Transmission electron microscope (TEM) images were obtained from JEM-2100 (JEOL, Japan) with a 200 kV accelerating voltage. The FT-IR spectra were obtained through a Bruker IFS 66 v/s infrared spectrometer.

2.4. Fluorescence detection of Hg^{2+}

Hg^{2+} detection was performed in a 60 μL mixture containing 1 × T7 exonuclease buffer (50 mM KAc, 20 mM Tris-HAc, 10 mM $MgAc_2$, 1 mM dithiothreitol, pH 7.9), 0.3 U μL^{-1} T7 exonuclease, 80 nM Probe 2, 25 nM Probe 1, and various concentrations of Hg^{2+} at 25 °C for 2 h, then 20 μL 50 μg mL^{-1} prepared single-layer WS_2 nanosheets solution and ultrapure water were added into the reaction solution with a final volume of 200 μL. The reaction was then incubated at room temperature for 5 min before fluorescence assay. For the selectivity evaluation, the fluorescence response of 20 nM