Analytical Methods

PAPER

A simple and sensitive colorimetric method for detection of mercury ions based on anti-aggregation of gold nanoparticles†

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A simple and sensitive method for the colorimetric detection of mercury ions (Hg^{2+}) has been proposed by using anti-aggregation of gold nanoparticles (AuNPs) based on the co-ordination between thymine and mercury ions. The thymine can bind to the AuNPs through Au–N bonds and induce aggregation of AuNPs. In the presence of Hg^{2+}, the thymine was released from the surface of AuNPs *via* the formation of a thymine-Hg^{2+} coordination complex, leading to the dispersion of AuNPs. The detection reagent can be simply prepared by mixing thymine with citrate-capped AuNPs. This method is not only cost-effective, but also avoids complicated surface modifications and tedious separation processes.

Introduction

Mercury is one of the most potently toxic heavy metals which is widespread in the environment and has a severe adverse effect on human health.[1] Mercury ions (Hg^{2+}) are one of the most usual and stable forms of mercury pollution. Therefore, monitoring Hg^{2+} in the aqueous environment has received an increasing interest. Recently, a number of highly sensitive and selective methods for sensing Hg^{2+} have been developed, based on gold nanoparticles (AuNPs),[2] fluorophores,[3] DNAzymes,[4] polymer-materials,[5] proteins[6] and so on.

AuNPs-based sensing methods have attracted more and more attention due to their intrinsically high sensitivity and easy colorimetric read-out. Hence, various colorimetric detection methods based on surface modifications of AuNPs have been developed to selectively recognize Hg^{2+} in aqueous solutions.[7] Among these sensors, many are constructed with thymine (T) containing oligonucleotides as the sensing element. It is well-known that Hg^{2+} can bind with two T residues of DNA to form the T–Hg–T complex.[8] Only Hg^{2+} ions and no other metal ions have been found to bind with the T–T mismatch in duplex DNA to form T–Hg–T which can stabilize the duplex with the T–T mismatched base pairs and be able to direct the folding of single-stranded DNAs into duplexes.[9] It provides a rationale for applying T-containing oligonucleotide sequences for specifically sensing aqueous Hg^{2+} in diverse ways. Hence, a variety of colorimetric sensors based on T-containing DNA/AuNPs have been developed for the selective detection of Hg^{2+}. For example, Mirkin's group has reported a method based on the Hg^{2+}-induced aggregation of thiolated-DNA functionalized AuNPs based on T–Hg–T coordination chemistry[10] and complementary DNA–AuNPs with deliberately designed T–T mismatches;[11] Liu's group improved this strategy by using a different design, the presence of oligonucleotide-tethered gold nanoparticle probes and a linker oligonucleotide with a number of T–T mismatches results in the formation of particle aggregates with a concomitant colorimetric response after introduction of Hg^{2+} into the solution at room temperature.[12] Yang and co-workers also developed a method based on the fact that complementary DNA strands with T–T mismatches could effectively protect AuNPs from salt-induced aggregation, while the Hg^{2+} could weaken this protective effect by forming the T–Hg–T complex, and AuNPs are less well protected thus aggregate at the same salt concentration, accompanied by a color change from red to blue.[13] Although the developed sensors based on DNA/AuNPs possess good sensitivity and selectivity, the chemical synthesis and modification of DNA are relatively expensive and complicated. Additionally, the low stability against nuclease, and the nonspecific interaction resulting from the negatively charged backbone also limit the application of DNA-based sensors in real complex samples. All of these methods, however, still require the design and synthesis of various sophisticated DNA oligomer probes, which is tedious and expensive.

To reduce the operational cost and improve the oligo-nucleotide-based probe designs based on T–Hg–T coordination chemistry, some studies on the use of small thymine derivatives have been reported recently.[14] Liu and co-workers designed

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a colorimetric sensor based on thymine derivatives (thymine acetamidoethanethiol) modified AuNPs for Hg^{2+} detection with high selectivity.[15] We also designed a colorimetric method based on N-1-(2-mercaptoethyl) thymine modified AuNPs for detecting Hg^{2+} sensitively and selectively in our lab.[16] However, the above methods still need molecular modification of thymine and involve synthetic steps.

Here, we present a very simple and sensitive method based on the thymine molecule without any modification for the colorimetric detection of Hg^{2+}. In Hg^{2+}-free solution, thymine can be bind to the surface of AuNPs via Au–N bonds and exchange citrate ions, thus inducing the aggregation of the AuNPs accompanied by a color change from red to blue. While in the presence of Hg^{2+}, thymine in the solution can interact with Hg^{2+} and form T–Hg complexes, thus thymine cannot access the surface of the AuNPs and the color of solution is still red. This new type of unmodified AuNP-based chemosensor for Hg^{2+} ions in aqueous media possesses high sensitivity and selectivity. Compared to other approaches sensing Hg^{2+} based on thymine derivatives, our proposed method has many advantages: firstly, the sensing platform is label-free and does not need any modification of the AuNPs; secondly, the sensing system does not require any additional salt to induce a color change or aggregation of AuNPs; moreover, the method involves the use of small organic molecules that are stable and inexpensive thereby avoiding the use of oligonucleotides (DNA or RNA) or other derivatives which are relatively expensive or complicated. The sensitivity and selectivity of the method were also investigated. Also the application of the method for the detection Hg^{2+} in practical water samples was demonstrated.

Experiment section

1. Chemicals and materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), thymine (T), and the used metal salts, including $Hg(NO_3)_2$, $CdCl_2$, $MnCl_2$, $CoCl_2$, $CaCl_2$, $AlCl_3$, $FeCl_3$, $Pb(Ac)_2$, $ZnCl_2$, $NiSO_4$, $MgSO_4$, $FeSO_4$, $CuSO_4$ and $Ba(NO_3)_2$, were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Beijing, China), and stock solutions of these salts were prepared by dissolving them in deionized water. AuNPs with a diameter of 13 nm were prepared using the trisodium citrate reduction method.

2. Apparatus

Solutions were prepared with deionized water (18.2 MΩ cm specific resistances) purified by a Cascada TM LS Ultrapure water system (Pall Corp., USA). UV-vis spectra were recorded on a Thermo Nanodrop2000C instrument (USA). Photographs were obtained with a Canon Powershot A630 digital camera. TEM analysis was performed on a 10 JEM-1230 electron microscope (JEOL, Ltd., Japan) operating at 100 kV.

3. Methods

3.1 Nanoparticle synthesis. Citrate-capped AuNPs were prepared by means of the chemical reduction of $HAuCl_4$ in the liquid phase. In brief, 100 mL aqueous solution of 1 mM $HAuCl_4$ was brought to boil with vigorous stirring in a round-bottom flask fitted with a reflux condenser. 38.8 mM trisodium citrate (10 mL) was then added rapidly to the solution, and the mixture was heated under reflux for another 15 min, during which time its color changed from pale yellow to wine red. The solution was cooled to room temperature while being stirred continuously. The size of the citrate-capped AuNPs determined by TEM image was about 13 nm. The particle concentration of the gold colloid (ca. 15 nM) was determined according to Beer's Law.

3.2. Sample preparation. The detection was performed in 1 mM Tris-HCl buffer solution at different pH. For Hg^{2+} sensing, the ions were added to a buffer solution (800 μL) containing AuNPs (100 μL, 15 nM), and then thymine (100 μL, 0.05–0.8 mM) was added to the solution. After equilibrating at ambient temperature for the optimum incubation time, the absorption spectra of the resulting solutions were recorded .

3.3 Analysis of real samples. A series of samples were prepared by spiking standard solutions of Hg^{2+} to tap water obtained locally. These spiked samples were added to a buffer solution containing AuNPs, then different concentrations of thymine were added into the solutions and the solutions were incubated for some time before spectral measurement.

Safety considerations. As Hg^{2+} and most of the tested metal ions are highly toxic and have adverse effects on human health, all experiments involving heavy metal ions and other toxic chemicals should be performed with protective gloves. The waste solutions containing heavy metal ions should be collectively reclaimed to avoid polluting the environment.

Results and discussion

1. Sensing mechanism

The sensing mechanism of the anti-aggregation of the AuNPs sensor for Hg^{2+} is shown in Scheme 1. The citrate ions which keep AuNPs from aggregation on the surfaces are easily displaced by other ligands with heterocyclic N and SH groups.[17,18] The N-donors have a stronger affinity with AuNPs than the carboxyl
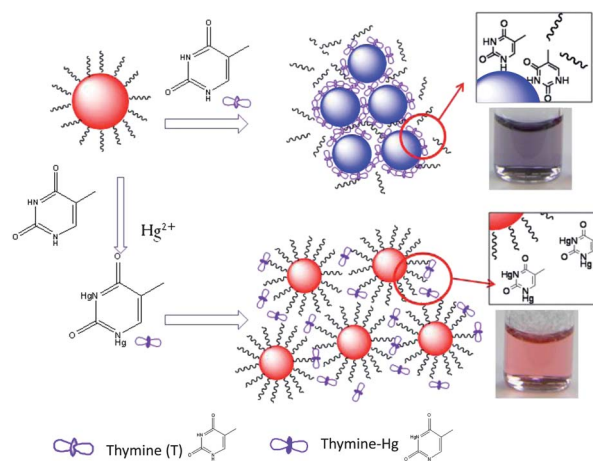
Scheme 1 A schematic mechanism of sensing Hg^{2+} in aqueous solutions based on anti-aggregation of gold nanoparticles.
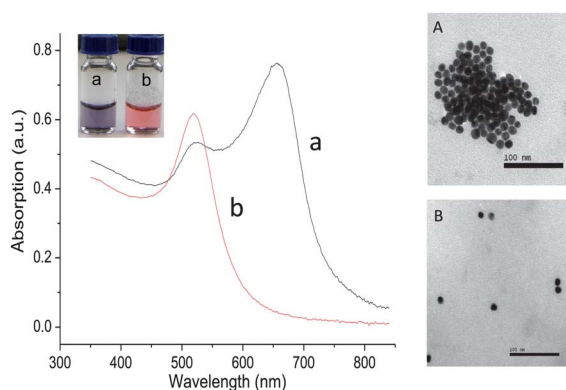
groups[19] because compounds with electron-rich nitrogen atoms easily bind onto the surface of metal nanoparticles through coordinating interactions between nitrogen atoms with the electron-deficient surface of the metal nanoparticles; in particular, the ring nitrogen of hybrid aromatics exhibits a much stronger binding ability/affinity to AuNPs.[19] When thymine was added into the AuNPs solution, the citrate ions on the surface which protects them from aggregation were replaced by thymine. The AuNPs could not stabilize and the color turned blue. However, when Hg^{2+} was present in the solution, the Hg^{2+} reacted with thymine and formed a T–Hg complex through N–Hg bonds like T–Hg–T. In that case, the thymine does not attach to the surface of AuNPs and the solution remains red.

The Hg^{2+}-induced anti-aggregation of AuNPs was further confirmed by UV-vis absorption and TEM images. As shown in Fig. 1, AuNPs aggregated after the addition of thymine in the absence of Hg^{2+} ions (Fig. 1, image A), which resulted from the replacement of citrate to thymine from the surface of the citrate-capped AuNPs. While in the presence of Hg^{2+} ions, AuNPs were well dispersed because the thymine could form complexes with Hg^{2+} ions and could not access the surface of the AuNPs. (Fig. 1, image B). The TEM images were consistent with the UV–vis absorption spectra as well as the color change of the solution in the absence and presence of 10μM Hg^{2+} ions, thus the designed sensing system was suitable for Hg^{2+} detection.

2. Feasibility for the detection of Hg^{2+}

The feasibility of using the sensing platform for the colorimetric detection of Hg^{2+} is demonstrated in Fig. 1. When a certain concentration of thymine was added into the solution, the citrate on the surface of AuNPs was replaced by thymine. Hence the AuNPs aggregated and the color changed to blue. A significant red shift of the surface plasmon was observed due to the aggregation of the AuNPs. A characteristic absorbance peak at about 670 nm was observed, as shown in Fig. 1. If 5 μM Hg^{2+} was present in the solution, with the subsequent addition of thymine, the solution was still red, and an absorbance peak was still present at about 520nm as shown in Fig. 1.

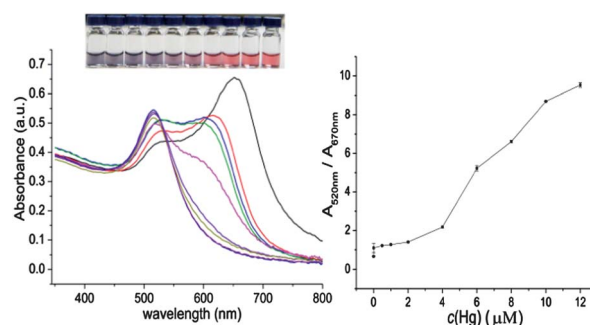We then explored the effect of pH on the Hg^{2+}-induced colorimetric response of the system in Tris-HCl buffer. The absorption ratio was maximized at pH 8.0 of the Tris-HCl (Fig. S1, ESI†). Next, the reaction time was investigated. The relative absorption intensity increased as the incubation time was extended. It was observed that after 30 min, the increase of the relative absorption was flattened. Therefore, an incubation time of 30 min was selected (Fig. S2, ESI†). The UV-Vis absorption spectra of AuNPs in Tris-HCl buffer solution at pH 8.0 with varying thymine concentrations ranging from 0.05 mM to 0.8 mM were recorded (Fig. S3, ESI†). If the concentration of thymine is low, the thymine in the colloid solution cannot induce the aggregation of AuNPs. However, the sensitivity is not good if the concentration of thymine is too high, and the remaining thymine which has not formed complexes with mercury ions could induce the AuNPs aggregation. Thus 0.2mM thymine was used for Hg^{2+} quantification.

3. Sensitivity of the sensor

To evaluate the sensitivity of the assay, different concentrations of Hg^{2+} from one stock solution were tested. The absorbance value of AuNPs solutions at 520 and 670 nm is related to the quantities of dispersed and aggregated AuNPs, respectively. Thus, the absorption ratio at 520 nm and 670 nm (A_{520nm}/A_{670nm}) was used here to reflect the ratio of dispersed and aggregated AuNPs. As shown in Fig. 2, the absorption ratio increased dramatically along with an increase in the Hg^{2+} concentration in the range of 5 nM to 12 μM, and a linear correlation was obtained over the range of of 2–12 μM ($R = 0.99$). A limit of detection of 2 nM was obtained for a signal-to-noise ratio of 3. When the Hg^{2+} concentration was above 12 μM, the absorption ratio reached a plateau, showing that almost all of the thymine had reacted with mercury ions to form T–Hg complexes. The corresponding colors of the solutions with different concentrations of Hg^{2+} are shown in the inset of Fig. 2. The solution color changed from blue to red gradually along with an increase in the Hg^{2+} concentration.

4. Selectivity of the sensor

To realize the selectivity of the system, some commonly co-existing metal ions were chosen for the investigation, including K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Al^{3+}, Pb^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}

Fig. 1 UV-Vis spectra (inset image is colorimetric response) and TEM images of AuNPs in the absence (a) and presence (b) of 10μM Hg^{2+} solution. (Scale bars: 100 nm).

Fig. 2 UV-Vis absorption spectra of AuNPs solution with different concentrations of Hg^{2+} (5 nM–12 μM) after the addition of thymine. The inset shows the corresponding pictures. The right shows a plot of A_{520nm}/A_{670nm} *versus* the concentration of Hg^{2+}. The error bars represent standard deviations based on three independent measurements.
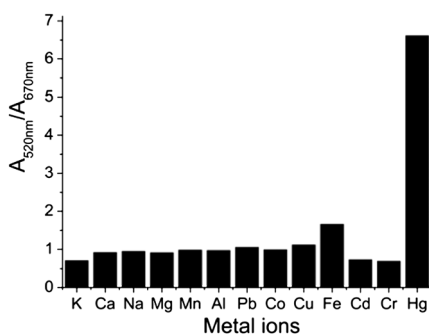
Fig. 3 The value of A_{520}/A_{670} of solution in the presence of 10 μM Hg^{2+} and 500 μM each of other metal ions including K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Al^{3+}, Pb^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+} and Cr^{3+}.

and Cr^{3+}. As illustrated in Fig. 3, only Hg^{2+} led to a significant increase in the absorption ratio after the addition of thymine, but other metal ions caused a slight increase. This indicated that only Hg^{2+} could induce the anti-aggregation of AuNPs solution because of the specific binding affinity of Hg^{2+} and thymine. Other metal ions cannot react with thymine to hamper the binding between thymine and AuNPs, with the consequence of aggregation of AuNPs. We had tested the tolerance above 500 μM and found that at concentrations above 1 mM of cations the stability of the AuNPs could be affected, causing the method to become invalid. So, it is particularly important to point out that the method is potentially applicable to soft natural waters only.

5. Determination of Hg^{2+} in real water samples

Considering the commonly concomitant alkaline earth and alkali metals, the matrix of tap water is more complex than other soft natural water matrices including river water and lake water (fresh). So, tap water sample spiked with Hg^{2+} was used to mimic the Hg^{2+} contaminated water for testing the practicability of this sensor. After the addition of tap water containing increased concentrations of Hg^{2+} to AuNPs solution, the solution color changed obviously from blue to red; the absorption ratio also increased gradually and reached a plateau at a concentration of Hg^{2+} above 10.0 μM. Additionally, the developed method provided recoveries of 88.0–112.5% of Hg^{2+} (see Table S1 ESI†), among which the two values significantly lower and higher than 100%, respectively, might result from some factors such as matrix effect and method errors, and are still acceptable. The results indicated the high potential of this anti-aggregation of AuNPs-based colorimetric method for Hg^{2+} quantification in aqueous solutions.

Conclusions

In summary, a very simple and cost-effective method based on the specific interaction of Hg^{2+} and thymine for the colorimetric detection of Hg^{2+} in aqueous solution was developed. Besides the inherent advantages of AuNPs-based assays, such as simplicity and high selectivity, this method offers other advantages: (1) The use of a simple and commercially available thymine as a Hg^{2+} acceptor avoids any design or molecular modification or

optimization of the Hg^{2+}-binding oligonucleotide. (2) This sensing platform is easily constructed in solution without any other labeling or modification steps. (3) The detection procedure is also rather simple: all it takes is recording the absorption spectrum or observing the color change after the addition of samples to the sensing system. This colorimetric sensor shows good potential for the visual detection of Hg^{2+} in complex samples.

Acknowledgements

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