Highly Sensitive Gold Nanoparticle-Based Colorimetric Sensing of Mercury(II) through Simple Ligand Exchange Reaction in Aqueous Media

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ABSTRACT A strategy for the rational design of a novel colorimetric sensor based on dithioerythritol-modified gold nanoparticles for the selective recognition of Hg^{2+} in aqueous media is presented. This approach relies on the combination of gold nanoparticles with Hg^{2+} through sulfur−Hg^{2+}−sulfur interaction. The gold nanoparticles showed high selectivity toward Hg^{2+} with binding-induced red shift in the absorption spectra, with no response to major interfering cations such as Pb^{2+}, Cd^{2+}, and Cu^{2+} in the presence of ethylenediamine tetraacetic acid. The system responds to Hg^{2+} with a detection limit of 100 nM and might open a new avenue for the development of Hg^{2+} sensing probes.

KEYWORDS: colorimetric • dithioerythritol • gold nanoparticles • ligand exchange • mercury

1. INTRODUCTION

Mercury is considered as one of highly toxic heavy elements that exists in metallic, inorganic, and organic forms. Mercury contamination is spread to the environment through anthropogenic sources, such as gold mining, the combustion of solid waste, fossil fuels, etc (1). Hg^{2+} shows strong affinity for the ligand containing sulfur atoms and causes the blocking of sulphydryl groups of proteins, enzymes, and membranes (2). Hg^{2+} is also related to the abnormal functions of brain, liver, kidney, and gastrointestinal tract (3). Because of its serious hazardous effects to human health, there is a strong need to develop a highly sensitive and selective sensor for measurement of Hg^{2+} in aqueous media (4).

A variety of detection techniques, such as atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and fluorescence spectroscopy have been developed for the determination of Hg^{2+}. These techniques require expensive instrumentation and complicated sample preparation processes in certain cases. Recently, a great effort has been exerted to develop Hg^{2+} sensors using organic chromophores (5), fluorophores (6, 7), oligonucleotides (8, 9), DNAzymes (10), conjugated polymers (11), microcantilevers (12), electrochemical methods (13, 14), and gold nanoparticles (AuNPs) (15). However, there are still major limitations associated with some of these sensors regarding their practical usage: solubility in aqueous media and poor sensitivity.

El-sayed and Link (16) have reported that AuNPs are important colorimetric materials because their absorption coefficients in the visible region are several orders of magnitude higher than those of common organic dyes. The colorimetric behavior of AuNPs is related to surface plasmon resonance (SPR). The SPR band is sensitive to the size, shape, and interparticle distance. AuNPs smaller than 60 nm in diameter exhibit an intense red color due to SPR absorption when their conducting electrons are confined to dimensions smaller than electron mean free path. When the aggregation of AuNPs takes place, the color turns blue or purple because of the coupling of the plasmon absorbance as a result of the nanoparticles coming in close proximity to each other. Using this concept, researchers reported AuNPs-based colorimetric recognition of DNA (17), proteins (18), and metal ions (19). In the case of metal ion sensors, the recognition properties depend on the functional groups on AuNPs. Ligand exchange reaction is one of the important processes to introduce the tailored functionality on AuNPs. In recent years, mercaptopropionic acid (20), mercaptoundecanoic acid (21), and thiol-modified 15-crown-5 (22) were used as capping reagents of AuNPs through ligand exchange reactions for the detection of Hg^{2+}, heavy metal ions, and K^+, respectively.

Hg^{2+} exhibits stronger thiophilic tendency compared with competing heavy metal ions such as Pb^{2+}, Cd^{2+}, and Cu^{2+} (23). Hence, many fluorescent receptors and chromophores for the detection of Hg^{2+} have sulfur-based functional groups (24−27). Encouraged by these observations, we envisioned that dithioerythritol (DTET) might be utilized for the modification of AuNPs; such an idea has been attempted in this
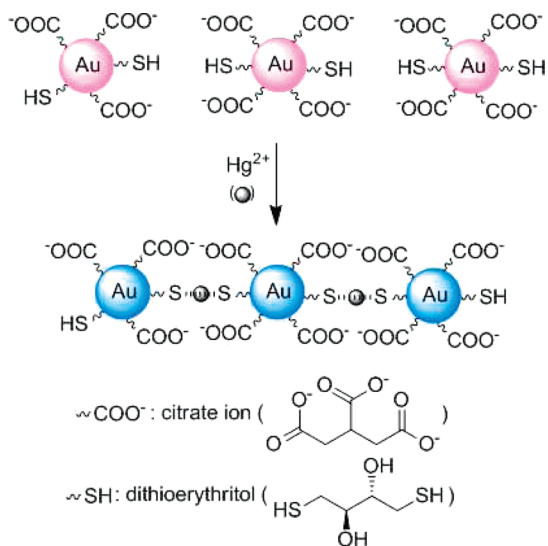
FIGURE 1. Mercury(II) sensing mechanism.

work for the development of Hg^{2+} sensor in aqueous media. We herein report on attractive sensor for Hg^{2+} detection through a specific sulfur−Hg^{2+}−sulfur interaction between dithioerythritol-modified AuNPs (DTET-AuNPs). Hg^{2+} leads to colorimetric change because of the change in SPR absorption. The Hg^{2+} sensing mechanism is shown in Figure 1.

2. EXPERIMENTAL SECTION

2.1. Chemicals. DTET was purchased from TCI (Tokyo, Japan) and used as received. Hydrogen tetrachloroaurate (III) trihydrate was purchased from Acros (Geel, Belgium). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) was purchased from Daejung Chemicals (Siheung, Korea). Sodium chloride, sodium citrate tribasic dihydrate, sodium hydroxide, and sodium phosphate monobasic were procured from Aldrich (Milwaukee, WI) and used as received.

2.2. Preparation of DTET-AuNPs. AuNPs were synthesized through the citrate-mediated reduction of $HAuCl_4 \cdot 3H_2O$ according to previous reported method (21). To an aqueous solution of $HAuCl_4 \cdot 3H_2O$ (100 mL, 1 mM) while boiling was rapidly added 10 mL of 38.8 mM sodium citrate under stirring. The color of the solution changed from pale yellow to deep red and was heated again for an additional 20 min. To the above gold suspension were added 5 mL of aqueous solution containing 50 mM DTET and 0.2 M sodium hydroxide. The mixture was stirred for 1 h and the solution was cooled to room temperature while stirring. Undersirable aggregates of DTET-AuNPs were removed by centrifugation (20 min, 3500 rpm) followed by the decantation of the solution. The DTET-AuNPs were obtained through centrifugation (30 min, 12 000 rpm) followed by decantation of the supernatants to remove excess of NaOH, citrate, and DTET. The solution of DTET-AuNPs was obtained by dissolving them in 200 mL of 10 mM phosphate buffer (pH 6.6; ionic strength 20 mM). NaCl was added to control the ionic strength of solution. The aggregates were finally removed by centrifugation (20 min, 3500 rpm).

2.3. Equipment. Transmission electron microscopy (TEM) measurements were made using a Hitachi H-7600 TEM at 100 kV. The average diameter of the DTET-AuNPs was found to be 16.0 ± 1.9 nm. The absorption spectra were obtained with a UV−vis absorption spectrometer (Scinco S-3100, Korea). The concentration of DTET-AuNPs in solution (ca. 8.8 nM) was determined according to the procedure described previously (28).
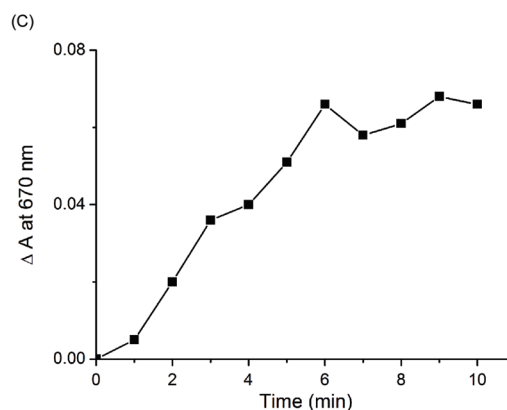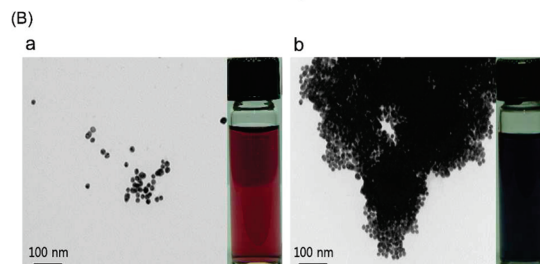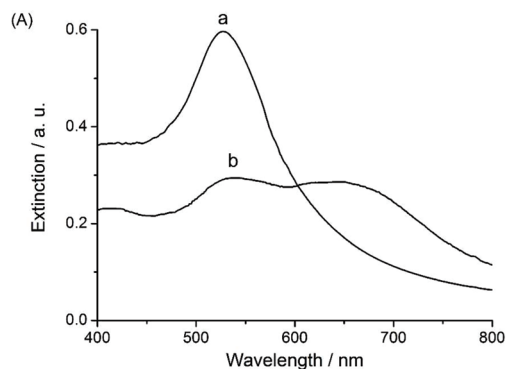

FIGURE 2. (A) UV−vis absorbance spectra; (B) photographic and TEM images of DTET-AuNPs solution in the (a) absence and (b) presence of Hg^{2+} (6 μM); (C) time-dependent changes of absorbance at 670 nm.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Responsive Condition of DTET-AuNPs to Hg^{2+}. It is well-known that AuNPs displayed a maximum absorption band at 520 nm. When AuNPs were modified by DTET, the absorption band shifted from 520 to 525 nm (Figure 2). The slight shift of wavelength may be related to the centrifugation step of the ligand exchanged AuNPs (29). Figure 2A shows the UV−vis spectra of a 8.8 nM suspension of the DTET-AuNPs. After the addition of Hg^{2+}, the spectrum exhibited a red shift with decreased absorbance, whereas the signal at 670 nm increased because of the aggregation of the DTET-AuNPs. These results are in agreement with Mie theory. According to Mie theory, when the distance between two nanoparticles becomes smaller than the sum of their radii, the SPR band displays a red shift, broadening, and decreased intensity (30). We confirmed that color change of DTET-AuNPs solution was induced by the addition of Hg^{2+}. The color changed immediately from red to purple upon the addition of Hg^{2+} (Figure 2B). Figure 2C shows that the Hg^{2+}-induced aggregation of the DTET-AuNPs reached its completion within 10
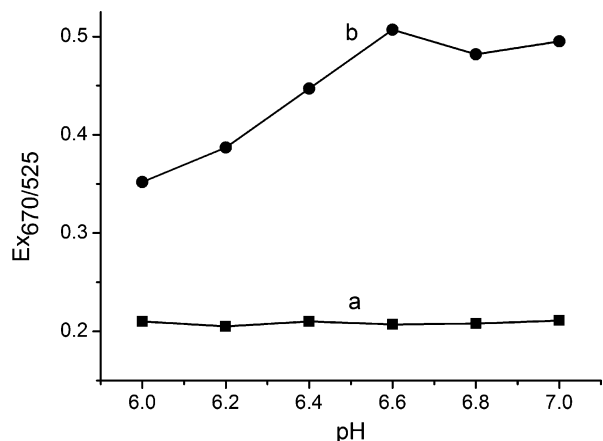
FIGURE 3. pH-Dependent changes of absorption coefficients ratio (Ex$_{670/525}$) in the (a) absence and (b) presence of Hg^{2+} (600 nM).

FIGURE 4. TEM images of DTET-AuNPs solutions containing (A) Hg^{2+} (1 μM) and (B) Hg^{2+} (6 μM) in the presence of EDTA (600 μM).

min. The solution became colorless after the completion of precipitation (see Figure S1A in the Supporting Information). TEM images also show that the aggregation of AuNPs occurred after the addition of Hg^{2+} (Figure 2B).

The pH and ionic strength of buffer are found to play important roles in the aggregation of AuNPs (22, 31). Hence, we investigated the effect of pH and ionic strength on the stability of DTET-AuNPs. When the pH is higher than 6.0, the DTET-AuNPs solution was stable for several days. However, if the pH is lower than 5.0, the precipitation of DTET-AuNPs solution occurred within several hours. To optimize the experimental condition, the values of colorimetric response (the ratio of absorption coefficients at 525 and 670 nm, Ex$_{670/525}$) were investigated around neutral pH (6.0–7.0). Figure 3 shows that pH 6.6 was found to provide the best performance of the colorimetric sensor. At pH 6.6, a negative charge density exists on DTET-AuNPs because the pK_{a3} of citrate is 6.4. Negatively charged citrate ions on the surface of AuNPs have a strong influence on the sensing of Hg^{2+} through cooperative effect and repulsion effect (19). The repulsion effect is the phenomenon in which the negative charge of citrate ions prevents the aggregation of DTET-AuNPs in the absence of Hg^{2+}. Furthermore, we found that 20 mM ionic strength was the most optimized condition for Hg^{2+}. Higher ionic strength (>30 mM) induced many aggregates and unstability in the synthesis of DTET-AuNPs process and not suitable for Hg^{2+} detection. As a result, in our experiments 10 mM phosphate buffer (pH 6.6; ionic strength 20 mM) was used.

3.2. Selective Detection of Hg^{2+} Using DTET-AuNPs.
To test the selectivity, the colorimetric responses in the presence of various metal ions including Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} at a concentration of 600 μM, 100 times greater than that of Hg^{2+}, were studied (Figure S1A in the Supporting Information). The DTET-AuNPs solutions did not exhibit any colorimetric response upon the addition of most interfering ions such as Pb^{2+} and Cd^{2+}. Only Cu^{2+} and Ba^{2+} led to the precipitation of the DTET-AuNPs after several hours. Thus, it is reasonable to believe that the rate of Cu^{2+} and Ba^{2+}-induced precipitation seems to be very slow. To further improve the selectivity
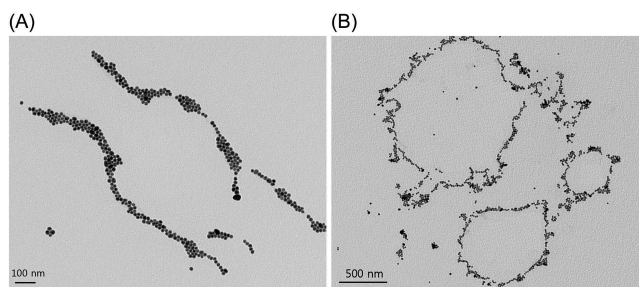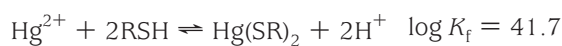
of the DTET-AuNPs toward Hg^{2+}, we added EDTA as a chelating agent, which exhibited effective masking ability toward Cu^{2+} and Ba^{2+} (Figure S1B in the Supporting Information). We also confirmed that the values of Ex$_{670/525}$ for many metal ions are quite small compared to Hg^{2+} (Figure S2 in the Supporting Information). The stronger affinity of Hg^{2+} toward two sulfur moieties of DTET-AuNPs than that of Hg^{2+} with EDTA is evident from the values of stability constant in following reactions (32, 33).

$$Hg^{2+} + 2RSH \rightleftharpoons Hg(SR)_2 + 2H^+ \quad \log K_f = 41.7$$

$$Hg^{2+} + EDTA^{4-} \rightleftharpoons HgEDTA^{2-} \quad \log K_f' = 17.6$$

Surprisingly, the structure of AuNPs aggregates changed to linear with 1 μM Hg^{2+} and ringlike with 6 μM Hg^{2+} following the addition of EDTA (Figure 4). On comparing with Figure 2B, we believe that the structural difference is due to the presence of EDTA, which leads to the segregation of the aggregates. It is well-known that AuNPs modified by carboxylic groups bind Hg^{2+} weakly (34). Hence, EDTA can lead to the extraction of weakly bound Hg^{2+} ions by citrate groups. The fact that DTET preferentially binds to the vertices and edges of AuNPs may be related to the orientation control of aggregates (35). Finally, repulsion between Hg^{2+} ions within the aggregates can be minimized in a linear formation, in which the Hg^{2+} ions are at the greatest distance away from each other. These structures are also related to formation of Hg(SR)$_2$ (36). This concept is similar to Hg^{2+} extraction experiment of Mirkin's group based on 2:1 complex between cysteine and Hg^{2+} (37).

3.3. Sensitivity of DTET-AuNPs to Hg^{2+}.
Figure 5A shows the colorimetric responses of the DTET-AuNPs upon the addition of various concentrations of Hg^{2+} in the presence of EDTA (600 μM). Above 600 nM Hg^{2+}, the colorimetric changes of the DTET-AuNPs solution were detected by naked eye. Figure 5B shows Ex$_{670/525}$ with various concentrations of Hg^{2+}. At high concentration (>600 nM) of Hg^{2+}, the solution became transparent due to the precipitation. As a result, Ex$_{670/525}$ was significantly decreased because many aggregates of DTET-AuNPs at high concentration of Hg^{2+} actually induced the decrease of absorption coefficient at 670 nm. At concentrations below 600 nM Hg^{2+}, a linear correlation ($R^2 = 0.9987$) exists between the value of Ex$_{670/525}$ and the concentration of Hg^{2+}. The concentration of Hg^{2+} up to 100 nM can be determined by the ratio of absorption coefficients. The relative standard derivations of triplicate measurements are 1.2%. The similar quantitative behavior has been reported by Lu et al. (38).
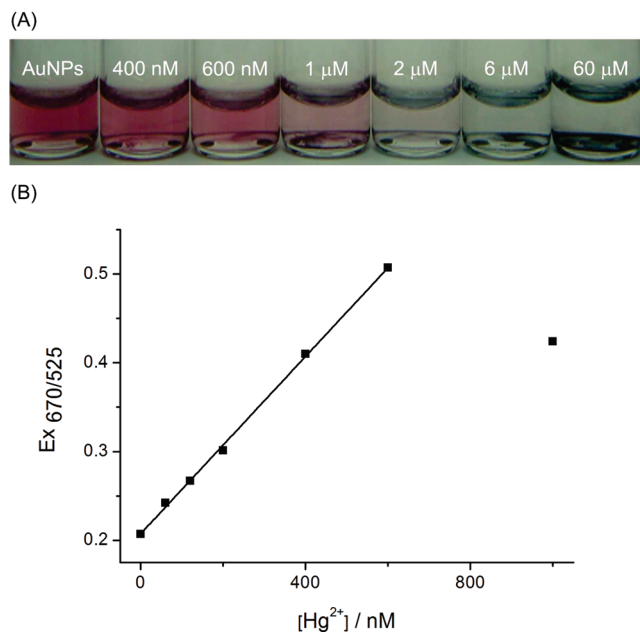
(A)

(B)

FIGURE 5. (A) Colorimetric responses and (B) Ex$_{670/525}$ of solutions containing DTET-AuNPs upon the addition of various concentrations of Hg^{2+} in the presence of EDTA (600 μM).

CONCLUSIONS

In summary, we have developed a colorimetric Hg^{2+} sensing system using DTET-AuNPs in aqueous media. The quantitative determination of Hg^{2+} can be made from color change of the solution as well as the ratio of absorption coefficients. This simple, rapid, and reliable sensing system provides additional advantages: it does not require a fluorescence dye, temperature control unit, and DNA engineering. Our efforts are currently underway to develop this sensor for separating Hg^{2+} by synthesizing DTET-modified gold coated magnetic nanoparticles.

Supporting Information Available: Colorimetric responses and the ratio changes of absorption coefficients of DTET-AuNPs solution after the addition of various metal ions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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