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Ultrasensitive Pb^{2+} Detection by Glutathione-Capped Quantum Dots

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Water-soluble and stable quantum dots (QDs), CdTe and CdZnSe, are applied for ultrasensitive Pb^{2+} detection. These QDs are capped with glutathione (GSH) shells. GSH and its polymeric form, phytochelatin, are employed by nature to detoxify heavy metal ions. As a result of specific interaction, the fluorescence intensity of GSH-capped QDs is selectively reduced in the presence of heavy metal ions such as Pb^{2+}. The detection limit of Pb^{2+} is found to be 20 nM due to the superior fluorescence properties of QDs. Detailed studies by spectroscopy, microscopy, and dynamic light scattering show that competitive GSH binding of Pb^{2+} with the QD core changed both the surface and photophysical properties of the QDs. Fluorescence of QDs is quenched, and QD aggregation occurs. Coupling the GSH-capped QDs with a high-throughput detection system, we have developed a simple scheme for quick and ultrasensitive Pb^{2+} detection without the need for additional electronic devices. In the presence of ionic mixtures, our system is still capable of Pb^{2+} detection with a detection limit as low as 40 nM. The system only becomes less sensitive when the ionic mixture is present at a very high concentration (i.e., ≥50 μM).

Contamination by heavy metal ions, particularly Pb^{2+}, poses a serious threat to human health and the environment.[1] Lead poisoning has been related to several diseases associated with environmental pollution.[2] The European Parliament is regulating lead usage in electronics to prevent hazardous chemical waste leaking to the groundwater.[3] The U.S. Environmental Protection Agency (EPA) set the safety limit of lead in drinking water as 15 μg/L. Because of health concerns and legal restrictions, it is critical to have probes that can provide rapid on-site evaluation of heavy metal contents. Toward this goal, various research groups have examined novel fluorescent probes that can selectively respond to Pb^{2+} over the past few years. These probes are either based on small organic luminescent dyes,[4,5] DNAzymes,[6] or metalloregulatory proteins.[7] However, they generally displayed detection limits around 10^{-1} μM and await further improvements on sensitivity and selectivity.

Recent advances in quantum dots (QDs) have shown great promise in molecular detection.[8−11] These nanocrystalline materials displayed superior luminescence properties and stability in aqueous solutions, and several groups have employed them as ion probes. Chen and Rosenzweig showed that fluorescence intensity of thioglycerol-coated CdS QDs was reduced selectively in the presence of Cu^{2+}.[12] Gattas-Asfura and Leblanc also described the optical detection of Cu^{2+} and Ag^{+} with peptide-coated CdS QDs.[13] However, no reports have shown responses of QDs toward Pb^{2+}. Mimicking nature, we reported the synthesis of glutathione (GSH)-capped CdTe and ZnCdSe with good quantum yields and long-term stability.[14,15] GSH plays an important role in heavy metal detoxification in cells in plants, yeasts, and bacteria, allowing the latter to grow in toxic soils. The physiological mechanism of detoxification involves the binding of heavy metal ion clusters by GSH, followed by metal−GSH complex polymerization to form metal sulfide−phytochelatin core−shell nanoparticles.[16] This natural phenomenon suggests that GSH or phytochelatin consists of the right shape and affinity for heavy metal ion binding. Both GSH and phytochelatin have been previously integrated into electrochemical systems for the detection of heavy metal ions.[17,18] Considering this, the competitive binding of free heavy metal ions such as Pb^{2+} should alter the surface structure of our GSH-capped QDs, changing the photophysical properties of the latter.

In this study, the GSH-capped ZnCdSe and CdTe QDs are demonstrated as selective fluorescent Pb^{2+} probes with low detection limit (20 nM). Integrated with microarray techniques,

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the GSH-capped QDs have been applied as rapid, convenient, and reliable assays for heavy metal ion detection.

EXPERIMENTAL SECTION

Materials and Reagents. All chemical reagents were purchased from Sigma-Aldrich, Merck, and Avocado, and used as received without further purification. The syntheses of GSH-capped CdTe and ZnCdSe QDs were described previously.[14,15] The QD stock solution was dialyzed to remove the remaining unbound GSH. Concentrated 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (1 M, pH 7.4) was purchased from Invitrogen. A 10 mM HEPES buffer solution was subsequently prepared and used as the medium for QD solutions.

High-Throughput Fluorescence Measurements. The QDs were dissolved in 10 mM HEPES buffer at pH 7.4. An amount of 75 μL of the buffer-diluted QD solution was mixed with 75 μL of cations of varying concentrations in a 96-well fluorescence plate using a Beckman Biomek NX multidispenser. The experiments were conducted with a freshly diluted QD solution, which was prepared prior to each experiment. The fluorescence intensity of the QDs under excitation at 345 nm was recorded by a microplate reader (Tecan Safire) within 5 min after the QDs were mixed with the ionic solution. Eight readings were taken under each experimental condition. The relative fluorescence unit was normalized with the background reading.

Selectivity Measurements. The following inorganic salts were used for the cation selectivity experiment: rubidium perchlorate, sodium nitrate, lithium perchlorate, cobalt(II) acetate, barium perchlorate, cesium perchlorate, calcium nitrate tetrahydrate, iron(III) sulfate pentahydrate, potassium nitrate, aluminum nitrate, nickel acetate tetrahydrate, zinc acetate, lead nitrate, and lead acetate. A 10 mM salt stock solution was prepared. Subsequently, salt solutions of 1 μM were prepared from the 10 mM stock solution by serial dilution. High-throughput screening was performed using a Tecan Safire^2 fluorescence microplate reader.

Fluorescence Quenching Measurements. Lead nitrate was used for the Pb^{2+} sensitivity studies. Various Pb^{2+} ion concentrations were prepared using serial dilution of the lead nitrate stock solution to test the sensitivity limits of the QDs. Pb^{2+} sensitivity plots of the QDs were obtained by methods adopted from high-throughput screening. Using the lead nitrate stock solution, Pb^{2+} concentration ranges of 0.05−20.0 μM and 0.005−1.0 μM were prepared. The QDs and the Pb^{2+} samples were mixed, and the resulting fluorescence reading was obtained using a Tecan Safire^2 fluorescence microplate reader.

Physical Characterization of Fluorescence Quenching with Pb^{2+}. Dynamic light scattering (DLS) measurements of GSH-CdTe QDs in aqueous solution were performed with a BI-200SM laser light scattering system (Brookhaven Instruments Corporation). Averages of five measurements were obtained. Mean-size displacement (MSD) volume was used as the output data. High-resolution transmission electron microscopy (HR-TEM) images of Pb^{2+}-treated GSH-CdTe QDs were collected with an FEI Tecnai TF-20 field emission HR-TEM (200 kV). Absorption spectra were obtained at room temperature with an Agilent 8453 UV−vis spectrometer. Fluorescence spectra were collected at room temperature on a Jobin Yvon Horiba Fluorolog fluorescence spectrometer. The QDs were excited at 345 nm.

An amount of 1 mL of 4 μM ZnCdSe (λ_{max} = 469 nm) was added to 1 mL of 0.1, 0.25, 0.5, and 1.0 mM Pb^{2+} ions. An amount of 1 mL of deionized water and 1.0 mM Ca^{2+} were used as controls. Three sets of samples were prepared. UV−vis absorption and fluorescence spectra were collected for the first set of samples. The second set of samples was used for DLS analysis and TEM studies. The third set of samples was placed in 7 mL glass vials, wrapped in aluminum foil, and kept in a 4 °C refrigerator for 24 h. A solution mixture of 40 μM GSH and 10 mM HEPES buffer was prepared. QDs were introduced to this solution mixture, which was used for the GSH interference study.

Interference Fluorescence Quenching Measurements. Dual ionic interference study was conducted by preparing a 1 μM solution mixture of Pb^{2+} and Rb^+, Li^+, Co^{2+}, Ba^{2+}, Cs^+, Ca^{2+}, Na^+, Fe^{3+}, or K^+. The QDs were mixed with the ionic mixture and characterized by high-throughput screening. For the ionic mixture interference study, 5, 10, and 50 μM Na^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Li^+, K^+, NO_3^-, Cl^-, ClO_3^-, CH_3COO^-, and PO_4^{3-} were prepared with 10 mM HEPES buffer. QDs were introduced to the ionic mixture, which was then mixed with various concentrations of Pb^{2+} ions and characterized by high-throughput screening.

RESULTS AND DISCUSSION

Selective Fluorescence Quenching of GSH-Capped QDs by Pb^{2+} Ions. We have previously reported the synthesis of GSH-capped QDs with a variety of core compositions in an aqueous environment.[14,15] These QDs were well dispersed in aqueous solution. They demonstrated high quantum yields and were successfully applied toward cell imaging. Herein, the selective fluorescence quenching experiments were performed with GSH-capped CdTe (GSH-CdTe, emission λ_{max} = 529 nm) and GSH-capped ZnCdSe (GSH-ZnCdSe, emission λ_{max} = 469 nm). Both QDs were 3 nm in diameter (Figure 1a), and emitted maximum fluorescence intensity by excitation at 345 nm. QDs of similar diameter were applied so that their responses could be compared, and they were surface-capped with a similar amount of GSH. The dialysis-purified QD solutions contained no free GSH. The luminescent intensity of these QDs were pH-dependent (Figure 1b). Therefore, 75 μL of GSH-capped QDs (4 nM) was first dispersed in HEPES buffer solution (10 mM, pH 7.4) and then added to the same amount of metal cation solutions (1 μM) in a 96-well plate. The fluorescence intensity of individual wells was measured with a fluorescence microplate reader. Figure 1c shows that both GSH-CdTe and GSH-ZnCdSe displayed no quenching response to alkaline and alkaline earth metal ions, Fe^{3+}, Al^{3+}, Ni^{2+}, and Zn^{2+}. In contrast, they demonstrated complete fluorescence quenching in the presence of Pb^{2+} ions. This selective response toward Pb^{2+} ions prompted us to conduct further studies on the fluorescence quenching.

Detection Limit for Pb^{2+} Detection. The detection of Pb^{2+} is of particular interest due to lead's environmental and biological toxicity. Figure 2 shows that GSH-ZnCdSe displayed greater fluorescence quenching at low Pb^{2+} concentration compared to GSH-CdTe. The Pb^{2+} detection limit of the more sensitive QD system was investigated further with GSH-ZnCdSe (λ_{max} = 499 nm) at different concentrations. We applied this QD system for

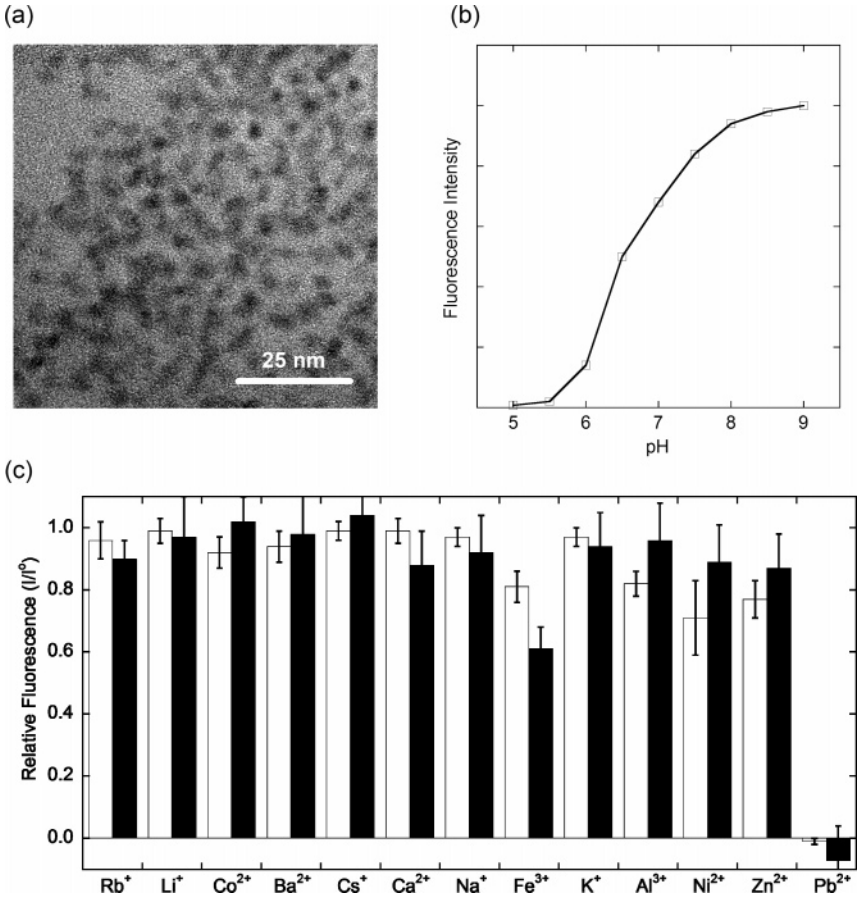

Figure 1. (a) Low-magnification TEM image of GSH-CdTe. (b) Fluorescence intensity of GSH-ZnCdSe (λ_{max} = 469 nm) in HEPES buffer at different pH values. (c) Effect of different ions on the fluorescence intensity of 4 nM (□) GSH-ZnCdSe (λ_{max} = 469 nm) and (■) GSH-CdTe (λ_{max} = 529 nm) in 10 mM HEPES buffer at pH 7.4. The excitation wavelength was 345 nm.

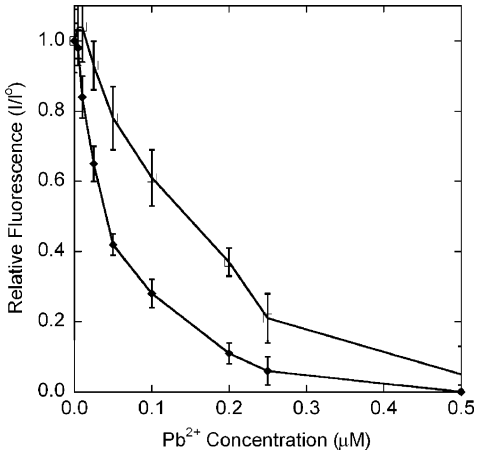

Figure 2. Effect of Pb^{2+} ion concentration on the fluorescence intensity of 4 nM (◆) GSH-ZnCdSe (λ_{max} = 469 nm) and (□) GSH-CdTe (λ_{max} = 529 nm) in 10 mM HEPES buffer at pH 7.4. The excitation wavelength was 345 nm.

more detailed studies because of its better long-term stability and shelf life. Figure 3a shows that greater fluorescence quenching was observed at lower GSH-ZnCdSe concentration in the presence of the same Pb^{2+} concentration. A concentration of 2 nM was the minimum QD concentration for Pb^{2+} detection by the fluorescence microplate reader with reasonable signal-to-noise ratio (S/N > 3). The measured fluorescence intensity was lower than the detection limit of the instrument for GSH-ZnCdSe solution concentration below 2 nM. Figure 3b illustrates that the GSH-ZnCdSe's response to Pb^{2+} was highly reproducible.

The fluorescence quenching was best described by the Stern−Volmer equation,

$$I^0/I = 1 + K_{SV}[Q] \quad (1)$$

where [Q] is the concentration of the quencher (i.e., Pb^{2+}) and K_{SV} is the Stern−Volmer constant. The linear relationship (R^2 = 0.996) of the Stern−Volmer plot of I^0/I versus Pb^{2+} concentration (Figure 3c) suggested that a single class of fluorophores was equally accessible to all the quenchers. It is important to note that $1/K_{SV}$ corresponds to the Pb^{2+} concentration when 50% of the fluorescence intensity is quenched. The linear relationship (R^2 = 0.997) between $1/K_{SV}$ and QD concentration as shown in Figure 3d suggested that we could lower the detection limit with diluted QD solution. Under the optimized condition, a 50% reduction in fluorescence intensity was observed for 2 nM GSH-ZnCdSe solution in the presence of 20 nM Pb^{2+} (i.e., $1/K_{SV}$ = 20 nM).

Mechanism of Pb^{2+} Detection by GSH-Capped QDs. The selective metal ion response (Figure 1c) of GSH-capped QDs is correlated to the relative metal−sulfide bond strength, determined

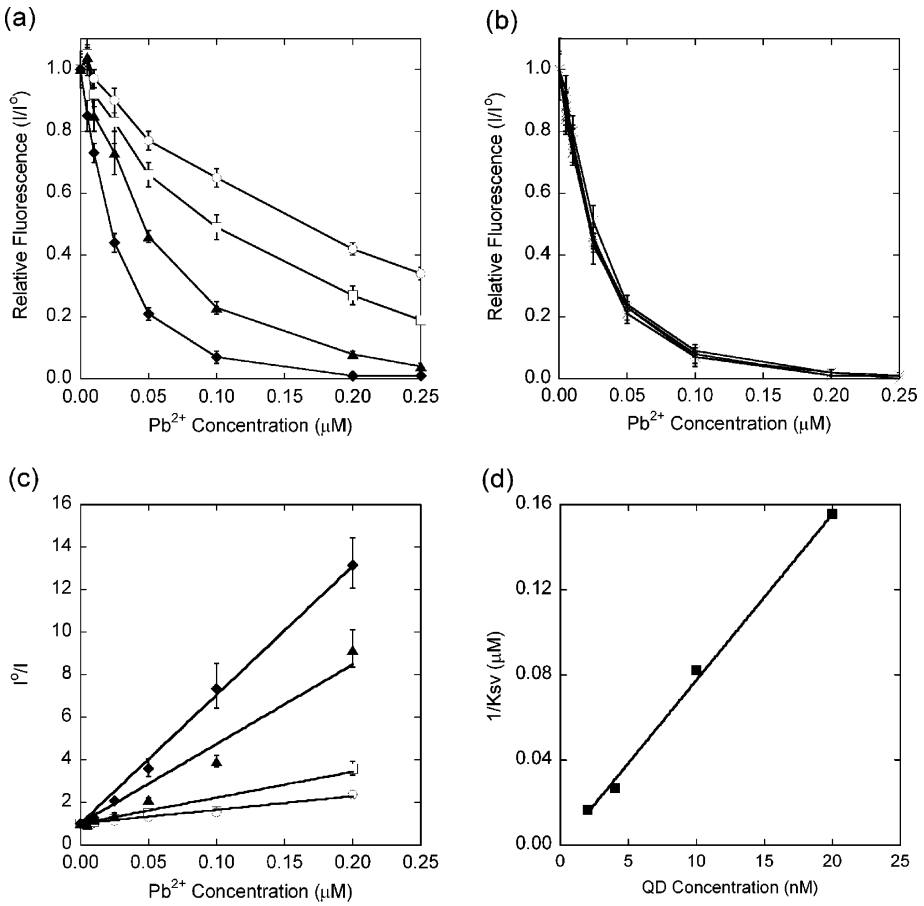

Figure 3. (a) Effect of Pb^{2+} ion concentration on the fluorescence intensity of (◆) 2, (▲) 4, (□) 10, and (○) 20 nM GSH-ZnCdSe (λ_{max} = 499 nm) in 10 mM HEPES buffer at pH 7.4. (b) Fluorescence quenching of six samples of 2 nM GSH-ZnCdSe (λ_{max} = 499 nm) in the presence of Pb^{2+} ions. (c) Stern−Volmer plot of (a). (d) Linear correlation of $1/K_{SV}$ values of GSH-ZnCdSe (λ_{max} = 499 nm) of different concentrations. The excitation wavelength was 345 nm.

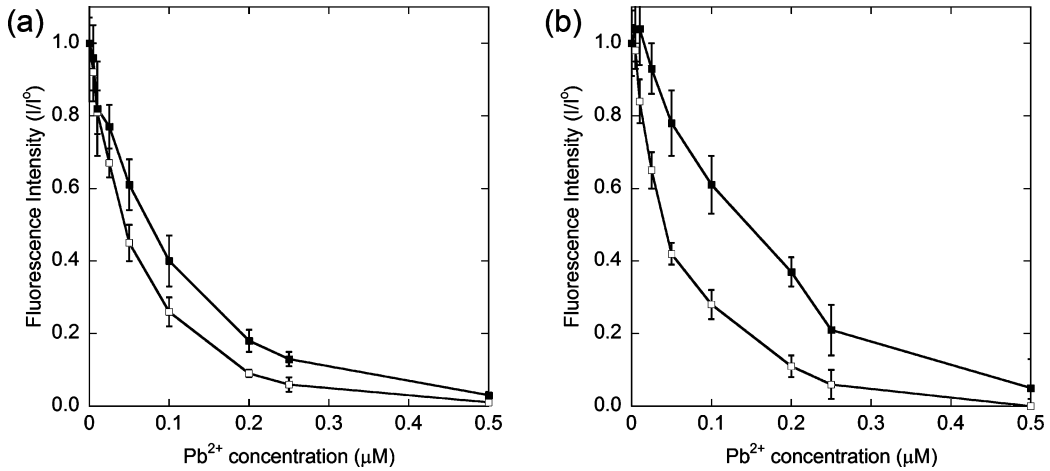

Figure 4. Fluorescence quenching by Pb^{2+} ions for 4 nM (a) GSH-ZnCdSe (λ_{max} = 469 nm) and (b) GSH-CdTe (λ_{max} = 529 nm) in 10 mM HEPES buffer solution at pH 7.4, in the (■) presence and (□) absence of 40 μM free GSH. The excitation wavelength was 345 nm.

by their respective K_{sp} value.[19] The GSH capping layer was very crucial toward the quantum yield and water stability of the QDs derived. The selective fluorescence quenching could be rationalized by the competitive GSH binding between the QD core and the metal ions present in the solution. The K_{sp} value of Pb−S (3×10^{-7}) is much lower than that of Zn−S (2×10^{-4}) and comparable to that of Cd−S (8×10^{-7}). Therefore, we proposed that the GSH capping was preferentially displaced from the surface of the QDs upon the binding of Pb^{2+} ions. This hypothesis was supported by the favorable binding of Pb^{2+} ion with the thiol group of the GSH peptide previously reported.[20] The displacement of GSH capping consequently created imperfections on the QD surface, resulting in fluorescence quenching. Greater fluorescence

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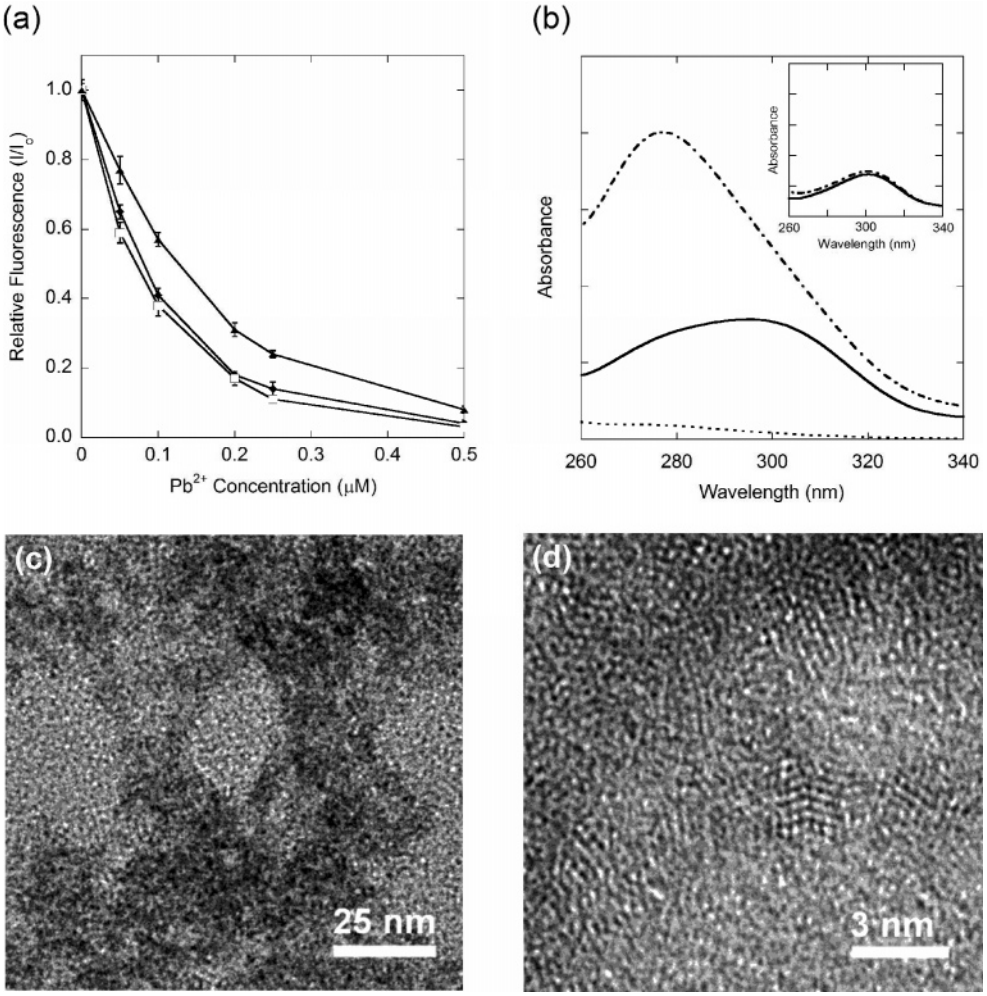

Figure 5. (a) Fluorescence quenching by Pb^{2+} ions for 10 nM GSH-ZnCdSe (λ_{max} = 499 nm) in 10 mM HEPES buffer solution at (◆) pH 5.2, (□) pH 7.4, and (▲) pH 8.8. The excitation wavelength was 345 nm. (b) UV−vis absorption spectra of (− − −) GSH, (—) Pb^{2+} ions, and (− · −) Pb^{2+} ions in the presence of GSH. Inset: UV−vis absorption spectra of (—) Al^{3+} ions and (− · −) Al^{3+} ions in the presence of GSH. The concentrations of GSH, Pb^{2+}, and Al^{3+} ions were all 20 nM. (c) Low-magnification and (d) high-magnification TEM images of GSH-ZnCdSe QDs in the presence of 1 mM Pb^{2+} ions.

Table 1. Summary of Spectroscopic and DLS Data of GSH-ZnCdSe QDs

ion concn	RFU (%)[a]	λ_{max} (abs) (nm)	λ_{max} (em) (nm)[b]	diameter (nm)[c]
0 mM Pb^{2+}	100.0	447	471	3.3[d]
0.10 mM Pb^{2+}	39.6	447	472	3.0[d]
0.25 mM Pb^{2+}	13.4	447	473	12.1[d]
0.50 mM Pb^{2+}	3.7	447	476	66.3[e]
1.0 mM Pb^{2+}	0.5	446	477	4799[e]
1.0 mM Ca^{2+}	96.0	448	471	4.5[d]

[a] Relative fluorescence unit. [b] Excitation at 345 nm. [c] Average particle diameter by DLS analysis. [d] Clear and homogeneous yellowish green solution after storage at 4 °C for 24 h. [e] Yellowish green precipitate noted at the bottom of the vial after storage at 4 °C for 24 h.

quenching in GSH-ZnCdSe QDs as compared to GSH-CdTe QDs under the same condition (Figure 2) could also be explained by the weaker Zn−S bond. To confirm our hypothesis, fluorescence quenching experiments were conducted in the presence of free GSH (Figure 4). Reduced fluorescence quenching in the presence of Pb^{2+} was observed in both GSH-ZnCdSe and GSH-CdTe QD solutions containing free GSH. These results suggested that the GSH capping of the QDs was related to the fluorescence quenching.

Our proposed fluorescence quenching mechanism was different from that suggested by other researchers.[21,22] It was reported that heavy metal ion displaced the Cd in the CdSe and CdTe QDs due to its higher binding affinity to Te and Se, as indicated by its lower K_{sp} values. Isarov and Chrysochoos investigated the quenching emission of CdS with copper ions[23] and found that copper ions formed ultrafine complexes of Cu_xS (x = 1, 2). To further understand the underlying mechanism in our system, we have employed a variety of analytical methods, including fluorospectrometry and DLS. In the reports by Chrysochoos[23] and Ren's groups,[22] a shift was noted in the absorption and emission spectra of the QDs upon metal ion displacement. In contrast, the fluorescence quenching of GSH-ZnCdSe was observed without any significant shift in the emission or absorption peak (see Table 1). Instead, aggregation of the QDs was found at high Pb^{2+} concentrations, leading to precipitation. QD aggregation was not

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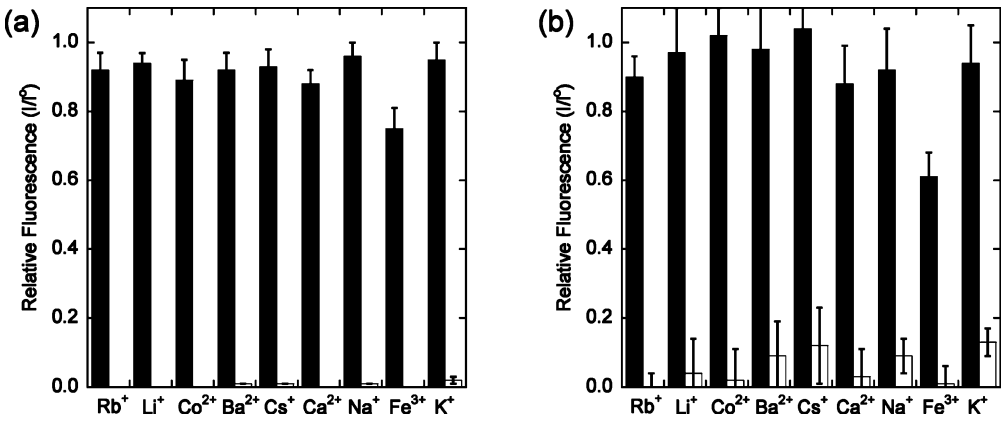

Figure 6. Response of 4 nM (a) GSH-ZnCdSe (λ_{max} = 469 nm) and (b) GSH-CdTe (λ_{max} = 529 nm) in 10 mM HEPES buffer solution at pH 7.4, in the (■) absence and (□) presence of 1 μM Pb^{2+} solution containing a specified interference metal ion of the same concentration. The excitation wavelength was 345 nm.

observed by DLS in the presence of 0.1 mM Pb^{2+} ions, despite a 60% reduction in fluorescence intensity. At a higher Pb^{2+} concentration of >0.25 mM, the fluorescence of QDs was further quenched and particle aggregation was observed. This indicated that the aggregation should be the result of surface ligand stripping, instead of the cause of fluorescence quenching. A control experiment with 1 mM Ca^{2+} ions confirmed that the aggregation and subsequent precipitation of QDs were induced by the Pb^{2+} ions and not by the increase in the solution's ionic strength. Another plausible explanation of the precipitate formation and fluorescence quenching was the coordination between Pb^{2+} and carboxylate of GSH. However, it would be difficult to rationalize the selective response toward Pb^{2+} based on this hypothesis. To further evaluate this hypothesis, the fluorescence quenching experiment was performed at different pH values. If the coordination of Pb^{2+} and carboxylate gave rise to the observed phenomena, the detection limit of Pb^{2+} would be pH-dependent. However, the fluorescence quenching was found to vary little between pH 5.2 and pH 8.8 (Figure 5a). Therefore, the fluorescence quenching was unlikely to be due to the carboxylate−Pb^{2+} coordination. UV−vis spectra of Pb^{2+}−GSH complex formation suggested that the selective reactivity to metal ions was associated with the GSH coating. A blue-shifted absorption peak (λ_{max} = 277 nm) with increased intensity was observed when Pb^{2+}−GSH complex was formed, as compared to Pb^{2+} (λ_{max} = 294 nm) or GSH solution alone (Figure 5b). The control experiment showed no spectroscopic difference for Al^{3+} solution in the presence and absence of GSH. Based on these findings, we concluded that the competitive binding of the GSH with Pb^{2+} was the primary mechanism for fluorescence quenching. The fluorescence intensity of QDs was highly sensitive to their surface protection. Even the removal of a limited amount of surface-bound GSH would lead to dramatic reduction in fluorescence. Therefore, the lower detection limit and higher sensitivity were achieved with the GSH-capped QDs. This would also explain why a decent sensitivity was still observed in the presence of a high concentration (40 μM) of free GSH. At higher Pb^{2+} concentrations, loss of more GSH would eventually cause the QDs to aggregate and precipitate. The low-magnification TEM image (Figure 5c) of ZnCdSe confirmed the aggregation of QDs. The high-magnification TEM image (Figure 5d) showed that the crystalline structure of the QD was not affected by the presence of Pb^{2+} ions.

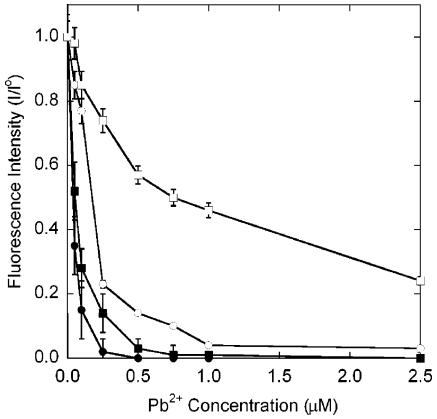

Figure 7. Effect of Pb^{2+} ion concentration on the fluorescence intensity of 2 nM GSH-ZnCdSe (λ_{max} = 499 nm) in 10 mM HEPES buffer at pH 7.4, in the presence of (●) 0, (■) 5, (○) 10, and (□) 50 μM ionic mixture solution. The ionic mixture consisted of Na^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Li^+, K^+, NO_3^-, Cl^-, ClO_3^-, CH_3COO^-, and PO_4^{3-}. The excitation wavelength was 345 nm.

Pb^{2+} Detection in the Presence of Other Metal Ions. To verify the performance of the GSH-capped QDs as a Pb^{2+} probe in real samples, Pb^{2+} detection was investigated in the presence of other metal ions (Figure 6). Both GSH-ZnCdSe and GSH-CdTe QDs were able to detect Pb^{2+} ions in the presence of other metal ions, without loss in sensitivity. Reduced sensitivity was only observed in the presence of a high concentration of ionic mixtures (see Figure 7). Detection of Pb^{2+} by GSH-ZnCdSe QDs was less sensitive ($1/K_{SV}$ = 0.78 μM) in the presence of an ionic mixture containing 50 μM Na^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Li^+, K^+, NO_3^-, Cl^-, ClO_3^-, CH_3COO^-, and PO_4^{3-} ions. However, in the presence of an ionic mixture containing only 5 μM of these ions, GSH-ZnCdSe QDs were able to detect Pb^{2+} ions with minimal reduction in sensitivity ($1/K_{SV}$ = 0.04 μM). The only ions that strongly interfered with the detection were Ag^+ and Cu^{2+} because they also showed a similar quenching effect as Pb^{2+} at similar concentration levels. This remains the limitation of our system despite its higher sensitivity and selectivity as compared to other QD-based metal ion detection systems.[12,13] The Cu^{2+} interference was most likely due to a different mechanism such as metal cluster

incorporation or coordination-mediated aggregation.[24] It might be eliminated with improved peptide coating. Currently, it would be necessary to pretreat the samples to avoid interference from Ag^+ and Cu^{2+} ions in the GSH-capped QDs detection scheme. Nevertheless, our system would provide for simple, sensitive, and high-throughput detection for on-site screening applications prior to more vigorous analysis.

CONCLUSIONS

GSH-capped QDs demonstrated selective fluorescence quenching in the presence of Pb^{2+} ions. Fifty percent of the fluorescence intensity of GSH-ZnCdSe QDs was quenched in the presence of only 20 nM Pb^{2+}. The fluorescence quenching can be attributed to the stronger binding between heavy metal ions and the surface GSH capping layer. By coupling the GSH-capped QDs with a high-throughput detection system, we have produced a simple tool for quick and ultrasensitive Pb^{2+} detection without the need for additional electronic devices. This nature-mimicking system is capable of Pb^{2+} detection even in the presence of an ionic mixture and only becomes less sensitive when the ionic mixture is present at a very high concentration.

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