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Ligand Replacement-Induced Fluorescence Switch of Quantum Dots for Ultrasensitive Detection of Organophosphorothioate Pesticides

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The development of a simple and on-site assay for the detection of organophosphorus pesticed residues is very important for food safety and exosystem protection. This paper reports the surface coordination-originated fluorescence resonance energy transfer (FRET) of CdTe quantum dots (QDs) and a simple ligand-replacement turn-on mechanism for the highly sensitive and selective detection of organophosphorothioate pesticides. It has been demonstrated that coordination of dithizone at the surface of CdTe QDs in basic media can strongly quench the green emission of CdTe QDs by a FRET mechanism. Upon the addition of organophosphorothioate pesticides, the dithizone ligands at the CdTe QD surface are replaced by the hydrolyzate of the organophosphorothioate, and hence the fluorescence is turned on. The fluorescence turn on is immediate, and the limit of detection for chlorpyrifos is as low as ~0.1 nM. Two consecutive linear ranges allow a wide determination of chlorpyrifos concentrations from 0.1 nM to 10 μM. Importantly, the fluorescence turn-on chemosensor can directly detect chlorpyrifos residues in apples at a limit of 5.5 ppb, which is under the maximum residue limit allowed by the U.S. Environmental Protection Agency. The very simple strategy reported here should facilitate the development of fluorescence turn-on chemosensors for chemo/biodetection.

About two million tons of organophosphorus (OP) pesticides are used annually to control insects in agriculture across the world but cause widespread contamination of air, water, soil, and agricultural products, eventually leading to long-term accumulation in ecosystems including humans.[1−4] The high toxicity of OP compounds results from irreversible binding to acetylcholinesterase and thus inflicts serious harm to the human nervous system, respiratory tract, and cardiovascular system. Nowadays, more than 200 000 people are estimated to die yearly from acute and chronic OP poisoning mainly due to food and drinking water contamination.[2] Therefore, there is a great demand to develop a fast, sensitive, and reliable assay of OP pesticide residues to improve food safety and to protect the ecosystem.

OP pesticides mainly include two kinds of compound derivatives, organophosphorus esters (containing P═O bond) and organophosphorus thioates (containing the P═S bond). Organophosphorothioate pesticides such as chlorpyrifos (CP) are of highest concern because of wide usage not only in agriculture (~33 000 tons in the U.S.) but also in nonagricultural areas (~8500 tons in the U.S.).[3,4] Moreover, relatively high environmental persistence causes organophosphorothioate residues to accumulate more easily in agricultural products and the environment. For example, the U.S. Environmental Protection Agency (EPA) has specially published the Report of CP Risk Assessments and has prescribed a maximum residue limit of CP in almost all agricultural products.[4] Although there is a wealth of established techniques for the assay of organophosphorothioate pesticides, such as chromatography−mass spectrometry[5] and enzyme-linked immunosorbent assay (ELISA) tests,[6] these analyses are usually carried out in well-equipped laboratories and involve complex sample preparation, expensive equipment, or costly biomolecular reagents. The fast/direct assay of ultratrace organophosphorothioate residues in complex matrixes such as environmental samples and agricultural products remains a great challenge.

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Recently, considerable effort has been devoted to develop chemosensors for the simple and on-site detection of OP pesticides by cooperative recognition and signaling, which have apparent advantages over conventional time-consuming off-line measurements. For example, cholinesterase or organophosphorus hydrolase has been used as a recognition element for selective binding, decomposition, and detection of OP pesticides.[7−10] On the other hand, various techniques using nanomaterials for the optical/electronic sensing of OP pesticides,[8−12] such as immunoassays based on gold nanoparticles,[9] electrochemical detection using carbon nanotubes[10] and molecularly imprinted nanoparticles,[11] and fluorescence quenching through enzyme-functionalized quantum dots,[12] have been explored. Of various chemosensory protocols, it is most conceivable to indicate the presence of an analyte by color or fluorescence changes that can be observed by the naked eye or by simple measurements. Recent advances in nanochemistry/optics have driven a growing interest in fluorescence turn-on sensors due to higher selectivity and sensitivity and a wider applicability.[13−18] Fluorescent quantum dot (QD) sensors are an extensively studied system, which operate by a mechanism of fluorescence resonance energy transfer (FRET).[16−18] Fluorescent QD sensors have been used for the assay of metal ions,[15] small molecules,[16] biomolecules,[17] and cells.[18] However, the assembly of FRET-based QD sensors is usually a complicated chemical process involving the modification of the QD surface, the immobilization of biological receptors, and the adsorption of a chosen dye quencher. Therefore, the QD fluorescence turn-on assay for the detection of analytes is achieved only under a harsh conditions and has rarely been reported for the detection of OP pesticides.

Herein, we demonstrate a surface coordination-originated FRET of CdTe QDs and a simple ligand-replacement fluorescence turn-on mechanism for the highly sensitive and selective detection of organophosphorothioate pesticides without the need for costly antibodies or enzymes. The fluorescent turn-on response is specific to organophosphorothioates and allows the detection of CPs as low as ~0.1 nM. More importantly, the fluorescence chemosensor can directly detect CP residues at 5.5 ppb, which is lower than the maximum residue limit of 10 ppb allowed by the EPA, in apple juice.

EXPERIMENTAL SECTION

Materials and Reagents. Chlorpyrifos (CP), parathion-methyl (PM), dylox (DL), mevinphos (MVP), profenofos (PF), ethoprophos (EP), 3-mercaptopropionic acid (MPA), and diethyl chlorothiophosphate were purchased from Sigma. Analytical grade tellurium powder, sodium borohydride ($NaBH_4$), cadmium chloride hydrate ($CdCl_2 \cdot 2.5H_2O$), dithizone (DZ), ethanol, and acetonitrile were used as received from Shanghai Chemicals Ltd. Ultrapure water (18.2 MΩ cm) was obtained from a Millipore water purification system. An ultrafiltration membrane (10 kDa molecular weight) was supplied from Millipore Corporation.

Synthesis of Diethyl Phosphorothioate (DEP). Two milliliters of diethyl chlorothiophosphate (12.6 mmol) and 1.6 g of sodium hydroxide (42.0 mmol) were dissolved in a solvent mixture of acetonitrile and water (20:18, v/v). After the solution was stirred at 30 °C for 12 h, the reaction mixture was acidified with HCl (6 M) and extracted with 30 mL of chloroform three times. The organic layer was collected and evaporated on a rotary evaporator. The residue was dried under vacuum to obtain a pale yellow oil (DEP).

Preparation of Cd Complexes and Characterization by Mass Spectrometry. Dithizone−Cd and DEP−Cd complexes were prepared by mixing an equivalent volume of aqueous $CdCl_2$ solution with a methanol solution of dithizone and DEP, respectively. Typically, $CdCl_2$ was dissolved in ultrapure water for the preparation of cadmium ion stock solution (5.0×10^{-4} M). A 0.1 mL amount of $CdCl_2$ stock solution and 0.1 mL of dithizone methanol solution (1.0×10^{-4} M) were mixed and adjusted to pH = 12 with tetramethylammonium hydroxide. The dithizone−Cd complex solution was obtained. Meanwhile, DEP−Cd complex solution was also prepared by mixing 0.1 mL of $CdCl_2$ stock solution with 0.1 mL of DEP methanol solution (1.0×10^{-4} M). The above mixtures were sprayed at a flow rate of 6.0 μL/min into a ProteomeX-LTQ mass spectrometer (MS) employing a regular electrospray ionization (ESI) source setup. The ESI-MS spectra of dithizone−Cd and DEP−Cd complexes were recorded.

Preparation of CdTe QDs. CdTe QDs were synthesized in the aqueous phase by two reaction steps according to the reported

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method with minor modifications.[19] Briefly, 0.0638 g of tellurium powder and 0.1 g of $NaBH_4$ were added to 10 mL of ultrapure water under nitrogen atmosphere to form a black mixture, which was then stirred for 8 h in an ice bath. After the black color disappeared and white $Na_2B_4O_7$ was produced, the supernatant containing NaHTe was separated from the mixture and used as the precursor for the preparation of CdTe QDs. A 0.2284 g amount of $CdCl_2 \cdot 2.5H_2O$ and 210 μL of 3-mercaptopropionic acid (MPA) were dissolved in 125 mL of ultrapure water, and the pH value of the solution was then adjusted to 9 with 1.0 M NaOH. The solution mixture was deoxygenated by bubbling nitrogen for at least 30 min. The freshly prepared NaHTe solution was transferred into the above mixture (final molar ratio: $Cd^{2+}/Te^{2-}/MPA = 1:0.5:2.5$) under nitrogen atmosphere, and the CdTe precursor was immediately formed, accompanied by a color change from colorless to orange. After being stirred for 20 min at room temperature, the mixture was refluxed to produce CdTe QDs with desired sizes by controlling the refluxing time. Green and red fluorescent CdTe QDs were obtained by refluxing for 1 and 48 h, respectively. The prepared CdTe QD solution was then illuminated for 24 h with a 15 W UV lamp under aerobic conditions to enhance the fluorescence quantum yield. The crude CdTe QD solution was purified by ultrafiltration to remove excessive thiols, free cadmium ions, and byproducts. The purified CdTe QDs were finally dissolved in water to form a stock solution for further use. The concentration of CdTe QD solution was estimated to be around 30 μM based on the band edge absorption and the empirical equation derived by Peng and co-workers.[20]

Preparation of CdTe QD Fluorescence Probe and Detection of Pesticides. To prepare the QD-based fluorescence probe, the fluorescence of the purified CdTe QDs was first quenched by surface grafting of a special bidentate ligand, dithizone (DZ), according to the following procedure. Two microliters of the purified CdTe QD stock solution was injected into 3 mL of 0.01 M NaOH solution, and then 30 μL of dithizone ethanol solution (1 mM) was mixed into the above basic QD solution. The solution mixture was shaken thoroughly at room temperature. The surface of the CdTe QDs was then coated by dithizone through the coordination between surface cadmium ions and dithizone molecules. Such a QD-DZ probe was used for the detection of pesticides. Three microliters of a known concentration of analyte in ethanol was injected into the QD probe solution. The fluorescence spectra were recorded using a 365-nm excitation wavelength. All fluorescence measurements were performed at room temperature under ambient conditions.

Measurement and Calibration of Chlorpyrifos Residues in Spiked Apples. The apple was chosen as the spiked sample to evaluate the QD-DZ probe for the detection of CP pesticide residues in actual fruits. Dilute CP methanol solutions with different concentrations were sprayed onto apples by an atomizer. After 2 days at room temperature, the edible parts of the apples were taken and crushed into a homogenate. Twenty grams of apple homogenate was mixed with 50 mL of acetonitrile in a 100 mL flask and shaken vigorously for 20 min. Then, insoluble draff was removed by a simple filtration. A control sample was also prepared following the same procedure.

The CP content in the apple juice was detected using the fresh QD-DZ probe. Typically, 1 mL of the QD-DZ probe was added to a quartz curvette and the fluorescence spectrum of the QD-DZ probe was recorded. Subsequently, 30 μL of the apple juice was injected into the QD-DZ probe solution, and the resulting fluorescence enhancement was measured. The quantity of CP in the apple juice was finally determined by a standard correlation curve that was obtained by adding the CP to the nonspiked apple juice and measuring the fluorescence enhancement.

The real quantity of CP residue in the spiked apples was calibrated by high performance liquid chromatography (HPLC) analysis. The apple juice obtained from the spiked apples by the above procedure was mixed with 5 g of NaCl and then transferred into a 100-mL separating funnel. After being shaken for about 3 min and keeping the funnel still for 10 min, the upper organic phase was collected and dried with anhydrous sodium sulfate. The organic solvent was evaporated to dryness under reduced pressure. The residue was redissolved in 0.5 mL of methanol, and the solution was filtered through a 0.22 μm membrane. HPLC analysis was carried out using methanol/water (9:1, v/v) as mobile phase at a flow rate of 1.0 mL/min. CP was monitored by an ultraviolet detector at 290 nm.

Instrumentation. Steady-state luminescence spectra were recorded using a Perkin-Elmer LS-45 luminescence spectrometer. The UV−visible absorption spectra were obtained with a Shimadzu UV-2550 spectrometer. The structures of the Cd complexes were determined with a ProteomeX-LTQ mass spectrometer employing a regular ESI source setup. All chromatographic measurements were performed using a Shimadzu CTO-10ASvp system equipped with an ultraviolet detector and a C_{18} column (250 mm × 4.6 mm i.d., 5 μm from Dikma). Photographs were taken with a Canon-350D digital camera.

RESULTS AND DISCUSSION

Mechanism of the CdTe QD Fluorescence Switch. Scheme 1 illustrates the fluorescence switch mechanism for the detection of organophosphorothioate pesticide. CdTe QDs with a green emission of 520 nm were synthesized in the aqueous phase according to the reported method with minor modifications.[19] The use of excessive $CdCl_2$ led to a relatively large amount of residual unsaturated Cd ions at the surface of the CdTe QDs. It has been documented that dithizone as a bidentate chelator can efficiently coordinate with many metal ions such as cadmium, lead, zinc, mercury, copper, etc.[21] When the QD aqueous solution was adjusted to high basicity (pH 12) with NaOH and then mixed with dithizone ethanol solution, the dithizone was thus bound onto the surface of the CdTe QDs through a surface coordinating reaction. Meanwhile, the green fluorescence of the CdTe QDs was quenched by a FRET mechanism due to the spectral overlap between the emission of the CdTe QDs and the absorption of the surface dithizone−Cd complex (see the following text). Upon the addition of CP pesticide into the strong basic system, CP molecules are rapidly hydrolyzed to diethylphosphorothioate (DEP) and trichloro-2-pyridinol

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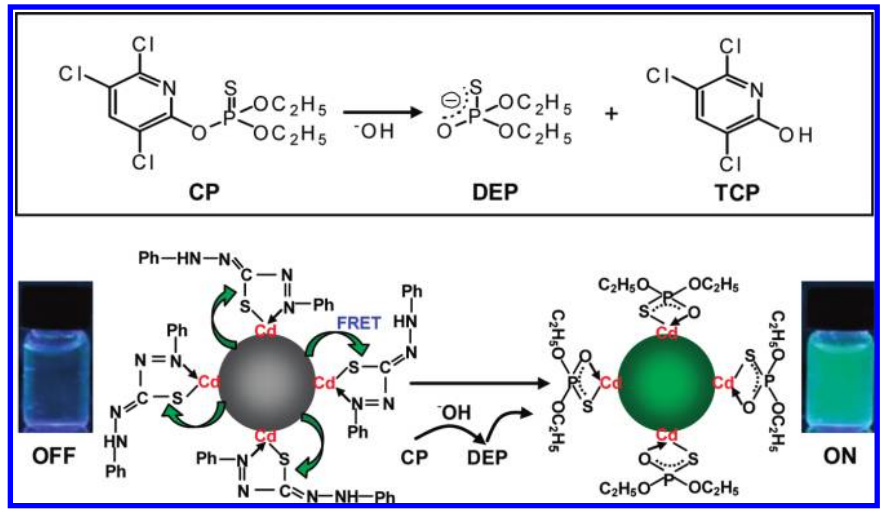
Scheme 1. Chlorpyrifos (CP) Is Hydrolyzed to Diethylphosphorothioate (DEP) and Trichloro-2-pyridinol (TCP) in Basic Media[a]



[a] The fluorescence of CdTe QDs is first quenched by the coordination of dithizone at the surface of CdTe QDs and subsequently turns on with the replacement of dithizone by DEP ligand.

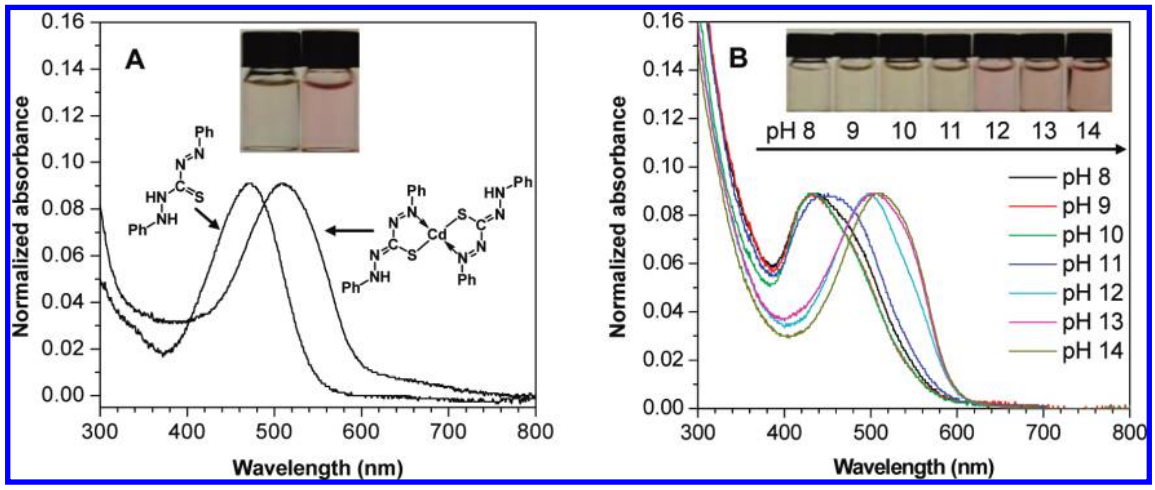


Figure 1. (A) Absorption spectra of dithizone and dithizone−Cd in 0.01 M NaOH solution (inset image shows the corresponding colors in natural light). (B) Evolution of absorbance spectra of the dithizone/CdTe QD mixture with pH value (2 μL of CdTe QDs is mixed with 10 μM dithizone. Inset image shows the corresponding color change with pH value).

(TCP).[22] The DEP moiety with a P═S bond replaces the dithizone ligands at the surface of the CdTe QDs due to its stronger coordinative interaction with metal ions.[23] The surface ligand replacement by DEP results in no effective spectral overlap between the absorption of the DEP−Cd complex and the emission of the QDs, because the DEP−Cd complex does not exhibit any visible absorption. Therefore, the fluorescence of the CdTe QDs is recovered by shutting off the FRET pathway, which allows for an ultrasensitive fluorescence assay of CP.

We substantiated the above assumption by detailed experiments involving absorption spectra, MS spectra, and fluorescence measurements. First, the coordinative reaction of dithizone at the surface of CdTe QDs can be clearly observed by absorption spectroscopy. As shown in Figure 1A, dithizone solution in 0.01 M NaOH shows a strong absortption at 470 nm. Upon the addition of Cd ions to the dithizone solution, the absorption shifts from 470 to 510 nm. Meanwhile, the color of the solution changes from colorless to light red, suggesting that dithizone can coordinate with Cd ions in the strong basic media (inset of Figure 1A). Furthermore, a similar reaction also occurs at the as-synthesized CdTe QD surface with rich residual Cd ions (Figure 1B). The mixture of dithizone and CdTe QDs at pH 8 shows an absorption peak centered at 450 nm. The increase of pH value from 8 to 14 leads to a large red shift from 450 to 510 nm in the absorption spectra, and the corresponding color gradually changes from colorless to light red (inset of Figure 1B). These above observations confirm that the dithizone molecules are bound to the surface of the CdTe QDs through the formation of a dithizone−Cd complex in strong basic media.

The coordinative reactions of Cd ions with dithizone and DEP and the resultant complex structures were further confirmed by ESI-MS spectroscopy. Figure 2 shows the ESI-MS spectra of the mixtures of Cd ions with dithizone and DEP in aqueous solution,

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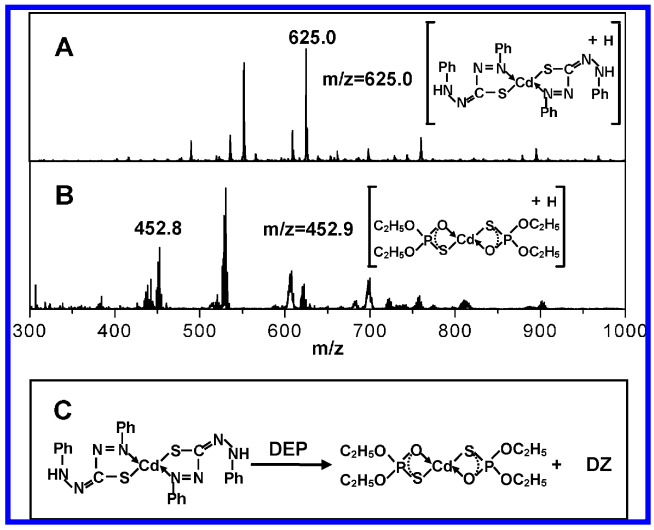

Figure 2. ESI-MS spectra of the mixtures of Cd ions with (A) dithizone (DZ) and (B) diethylphosphorothioate (DEP) in aqueous solution, respectively. (C) The replacement reaction from $Cd(DZ)_2$ to $Cd(DEP)_2$ complex.

respectively. After the Cd ions were mixed with dithizone in solution, the protonated $Cd(DZ)_2H^+$ peak at $m/z = 625.0$ was clearly detected (Figure 2A), suggesting the formation of dithizone−Cd complex in the mixture. On the other hand, the formation of the DEP−Cd complex was also confirmed by mass spectroscopy, which displays the peak of protonated $Cd(DEP)_2H^+$ peak at $m/z = 452.8$ when Cd ions were mixed with DEP in solution (Figure 2B). It is well known that CP is rapidly hydrolyzed to DEP and TCP in a strong basic media[22] (Scheme 1). However, the phosphorothioate-containing moiety due to the P═S bond exhibits a very strong coordinative ability with many metal ions and thus is widely used in floatation reagents of noble metals.[23] Accordingly, in the presence of DEP, the dithizone ligands at the surface of CdTe QDs are rapidly replaced by strongly binding DEP ligands to form a more stable complex (Figure 2C) because the P═S bond has stronger coordinative ability to metal ions than the P═O bond. This replacement was evidenced by the gradual decrease and blue shift of the 510-nm absorption of DZ-capped CdTe QDs with the addition of DEP (data not shown).

Figure 3 shows the normalized absorption spectrum of dithizone−Cd complex (black line) and the emissive spectrum of CdTe QDs (green line). Interestingly, the absorption of the dithizone−Cd complex (centered at 510 nm) completely overlaps the green emission of the chosen CdTe QDs (at 520 nm). As a result, the fluorescence of the CdTe QDs will be immediately quenched by the FRET from QDs to the surface dithizone−Cd complex. However, the DEP−Cd complex possesses an absorption spectrum (red line) very different from that of the dithizone−Cd complex. The absorption of the DEP−Cd complex exhibits a large blue shift with respect to the dithizone−Cd complex, and no visible absorption beyond 350 nm is observed. The surface replacement of dithizone by DEP results in no spectral overlap between the absorption of the DEP−Cd complex and the emission of the CdTe QDs, which is thus expected to shut off the pathway of FRET from CdTe QDs to the dithizone−Cd complex. As a result, the fluorescence of the CdTe QDs will be turned on with the addition

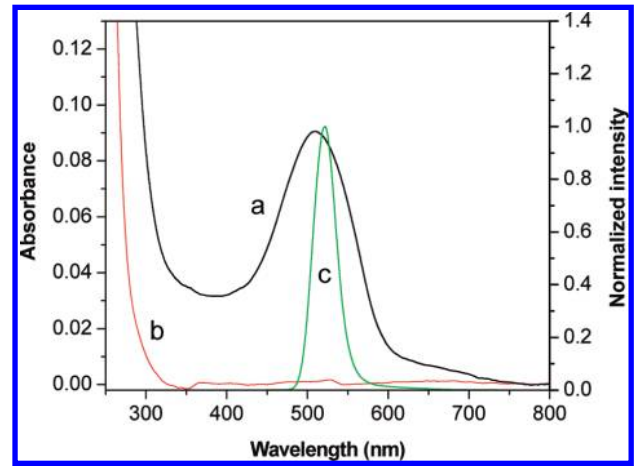

Figure 3. The absorption spectra of (a) dithizone−Cd and (b) DEP−Cd complexes and (c) the normalized emission spectrum of the CdTe QDs.

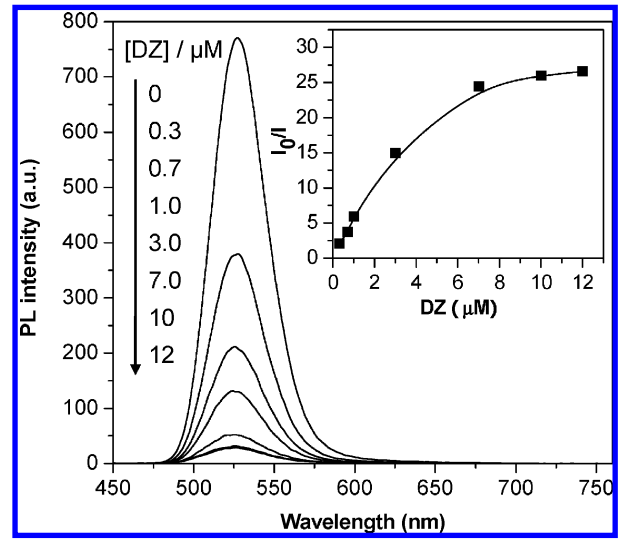

Figure 4. Fluorescence quenching of CdTe QDs (20 nM) upon the addition of dithizone. The inset shows the relationship between I_0/I and dithizone concentration (where I_0 and I are the fluorescence intensity of QDs in the absence and presence of dithizone, respectively).

of CP pesticide. The fluorescence enhancement can indicate the presence and quantity of CP pesticide in an ultrasensitive and specific way.

Fluorescence Switch for Detection of Organophosphorothioate Pesticide. Figure 4 shows the fluorescence quenching behavior by the formation of the dithizone−Cd complex at the surface of the CdTe QDs. The fluorescence intensity decreased gradually upon the addition of dithizone, and the fluorescence was nearly completely quenched at 12 μM. The quenching process follows a nonlinear behavior and shows a saturation concentration of dithizone (inset of Figure 4), indicating a static quenching mechanism and the formation of dithizone−Cd complex at the QD surface. The maximum emission wavelength and the shape of the emission spectra are still retained even at the lowest fluorescence intensity. This implies that the dithizone ligand cannot alter the size and size distribution of the QDs but can only reduce the fluorescence intensity through the FRET mechanism. The FRET mechanism-based quenching was further confirmed by a control experiment in which red-emissive CdTe QDs with a

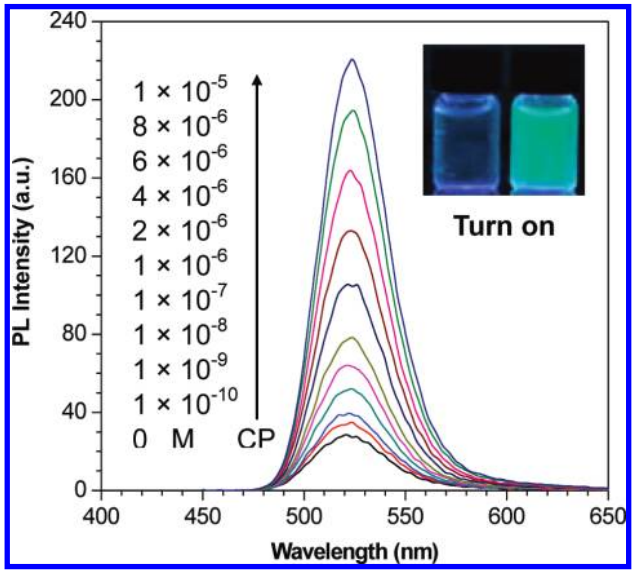

Figure 5. Fluorescence enhancement of the CdTe QD-DZ probe with the addition of CP (inset shows colorful images under a UV lamp before and after the addition of 10 μM CP).

maximum emission at 680 nm were used. The dithizone has no effect on the red fluorescence emission of the CdTe QDs (data not shown). Therefore, a QD-DZ probe can be constructed by the formation of the dithizone−Cd complex on the surface of green-emissive CdTe QDs. This QD-DZ probe shows extremely weak fluorescence intensity compared with that of the bare CdTe QDs at the same concentration level. Moreover, the weak fluorescence of the QD-DZ probe stays fairly stable over a relatively long time span and cannot be observed with the naked eye under a UV lamp (the image in Scheme 1). Thus, the fluorescence of the probe will be turned on if the surface dithizone ligands are replaced by the appropriate analyte, which is expected for ultrasensitive fluorescence detection.

With the addition of CP analyte to the CdTe QD-DZ probe solution, the fluorescence of QDs continuously recovers with the increase of CP concentration (Figure 5). Meanwhile, the solution changes from colorless to bright green under a UV lamp which can be seen with the naked eye (inset of Figure 5). About 8.5-fold fluorescence enhancement was measured when the concentration of CP reached 10 μM. Even at a concentration as low as 0.1 nM, fluorescence enhancement can be clearly observed with the fluorescence spectrometer, demonstrating an ultrasensitive response to CP pesticide. Similar results were obtained for other phosphorothioate pesticides such as parathion-methyl (PM) (data not shown). This is because phosphorothioate pesticides in basic media can all be hydrolyzed to DEP-like structures with a P═S bond. In the absence of dithizone ligands, however, the direct addition of CP or PM analyte to the pure CdTe QD solution with strong basicity does not result in any fluorescence enhancement and even causes a slight quench of the strong emission of the CdTe QDs. This reveals that phosphorothioate analyte and its hydrolyzates do not have any direct effect on the emission of pure CdTe QDs. The control experiments exclude the direct fluorescence enhancement by phosphorothioate and its hydrolyzates. This further confirms the fluorescence turn-on mechanism due to the surface ligand replacement as illustrated in Scheme 1.

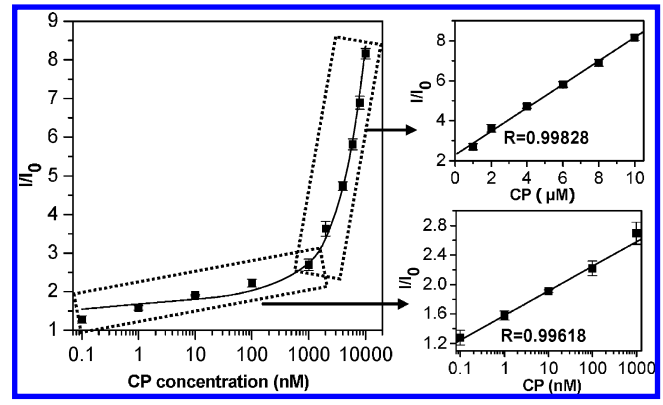

Figure 6. The plot of fluorescence enhancement vs CP concentration. The inserts on the right are linear correlations of the data included in the boxes.

Figure 6 shows the plot of fluorescence enhancement percentage vs the concentration of CP. Even if CP concentration was as low as 0.1 nM, the fluorescence intensity was still enhanced ~20%. Unambiguously, the fluorescence enhancement is closely related to the amount of CP added to the CdTe QD-DZ probe solution, which can be used for the quantification of CP analyte. With a further increase of CP concentration to 1 μM, the fluorescence was enhanced ~2.8 fold, and the enhancement was proportional to the CP concentration as shown by the highly linear calibration plot with standard deviation $R = 0.996$. When the concentration of CP was larger than 1 μM, the fluorescence was strongly enhanced and was also proportional to the CP concentration with $R = 0.998$. The evolution of fluorescence intensity is very suitable for the determination of CP within a very wide range of 0.1 nM to 10 μM in two consecutive linear ranges.

To better understand the mechanism of the fluorescent sensor, the fluorescence turn-on selectivity to various OP pesticides was compared. The chosen pesticides include six typical chemical structures, as shown in Figure 7. Only CP and PM with a phosphorothioate moiety (P═S bond) were able to turn on the fluorescence of the CdTe QD-DZ probe and resulted in a remarkable fluorescence enhancement. Although the other four pesticides can also be hydrolyzed into organophosphorus moieties with a P═O bond in basic media, these moeties are very weak coordinative ligands that are not able to replace dithizone at the surface of the CdTe QDs. Therefore, the additions of dylox (DL), mevinphos (MVP), profenofos (PF), and ethoprophos (EP) pesticides did not cause any change in fluorescent intensity from that of blank sample (Figure 7). Moreover, it is interesting that even the organophosphorus ester pesticides containing an $(RS)_n$ group, e.g., PF and EP, also did not result in any fluorescence enhancement of the QD-DZ probe. This unambiguously suggests that the P═S double bond, but not the P−S single bond or the P═O double bond, plays a key role in replacing dithizone to form a more stable complex at the surface of the CdTe QDs (Scheme 1). Therefore, the QD-DZ probe shows very high specificity for the detection of organophosphorothioate pesticides by the fluorescence turn-on mechanism through the surface ligand replacement. Note that the fluorescence turn on is slightly more sensitive to CP than to PM. This is attributed to either the difference of substituted groups in the phosphorothioate or the fluorescent quenching effect of the nitro group of PM. Meanwhile, the selectivity of fluorescence for CP was further confirmed by

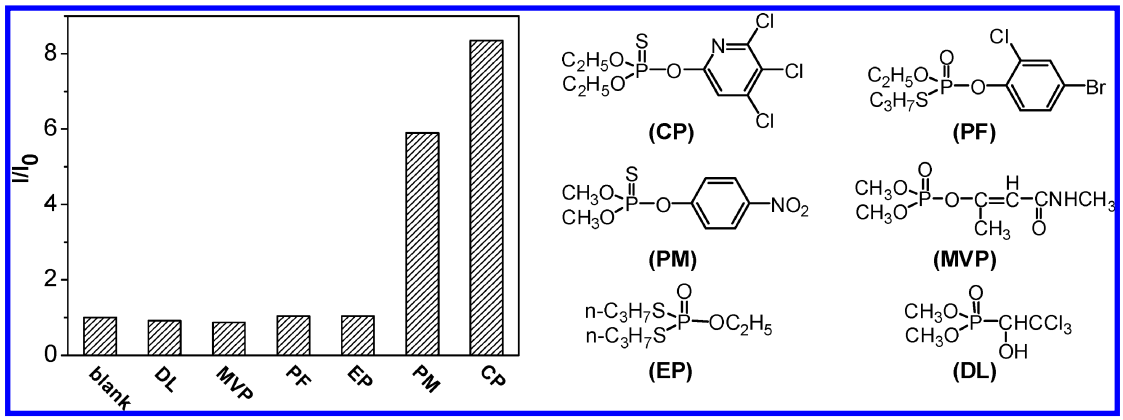

Figure 7. The fluorescence turn-on selectivity to various OP pesticides (10 μM) with [(RO)$_3$P=S] and [(RO/S)$_3$P=O] structures: chlorpyrifos (CP), parathion-methyl (PM), ethoprophos (EP), profenofos (PF), mevinphos (MVP), and dylox (DL).

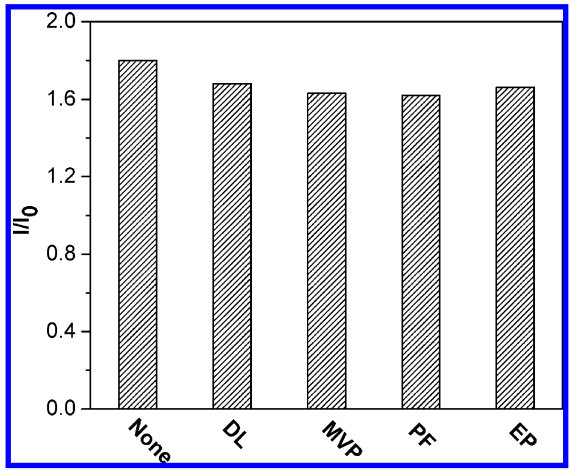

Figure 8. Fluorescence enhancement (I/I_0) of CdTe QD-DZ probe upon the addition of 0.1 μM of CP in the presence of other OP pesticides (10 μM).

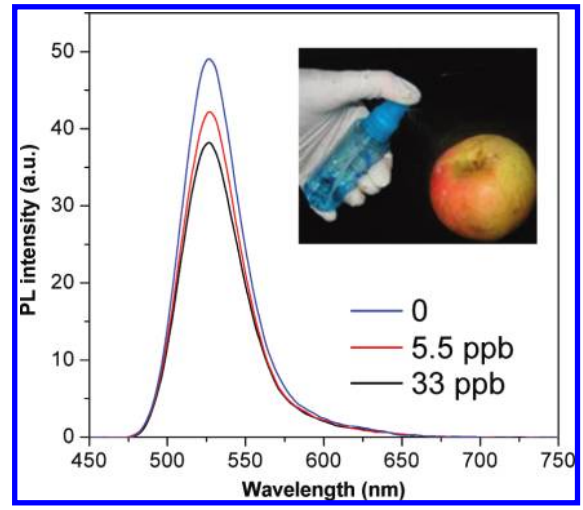

Figure 9. Fluorescence spectra before and after the addition of 30 μL of the juice from CP-spiked apples. The CP residues in two batches of apple samples are 6 and 25 ppb by HPLC calibration, respectively.

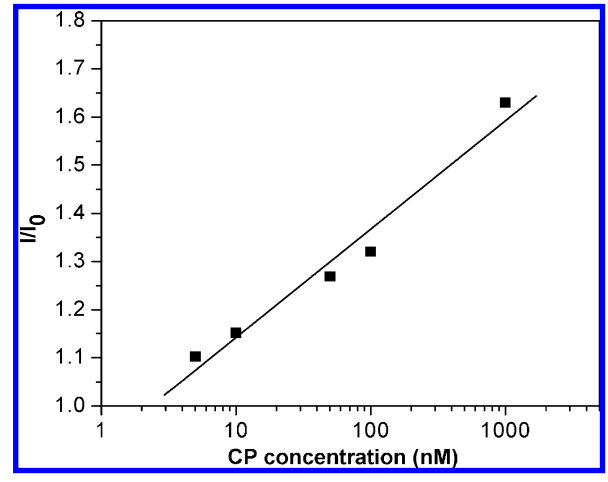

Figure 10. The standard curve of CP concentration in apple juice determined by the QD-DZ probe.

testing a mixture with four other organophosphorus ester pesticides, respectively. The coexistence of these pesticides even at 100-fold amount did not affect the detection of CP using the QD-DZ probe, as demonstrated by the interference experiments (Figure 8).

Detection of CP Residues in Spiked Apples. The utility of the QD chemosensor is largely dependent upon the direct detection of ultratrace CP residues in agricultural products. We chose apples as a test sample because the allowed CP residue limit in apples is the lowest (10 ppb) for all agricultural products and because the apple possesses many nutritive constituents including cellulose, sugars, vitamins, organic acids, minerals, polyphenols, and flavones. Dilute CP methanol solutions with different concentrations were sprayed onto apples by an atomizer, as shown in the inset image of Figure 9. After 2 days at room temperature, 20 g of edible flesh of the spiked apples was homogenated and mixed with 50 mL of acetonitrile. The mixture was shaken for 20 min and then filtered to remove the insoluble draff. Subsequently, 30 μL of filtrate was added to 1 mL of the QD probe solution. Figure 9 shows that two batches of samples from the spiked apples with different CP doses result in obvious enhancements of the QD fluorescence. The CP residues in the two batches of the spiked apples are calculated to be 5.5 and 33.0 ppb, respectively, based on the fluorescence signals and the standard curve (Figure 10). The values are very close to those calibrated by HPLC analysis (6 and 25 ppb, respectively), confirming the validation of the QD chemosensor in the detection of CP in real samples. The two amounts of CP residues are lower and higher than the maximum residue limit of 10 ppb in apples allowed

by EPA, respectively. Therefore, the sensitivity and anti-interference of the QD chemosensor should meet the requirements for direct detection of CP residues in agricultural products.

CONCLUSIONS

In summary, this work has demonstrated a surface coordination-originated FRET mechanism of CdTe QDs through the formation of a dithizone−Cd complex at the surface of the QDs, and a fluorescence turn-on mechanism through the replacement of dithizone ligands by the hydrolyzate of organophosphorothioate pesticides. These simple QD switch sensors have achieved fast/on-site ultrasensitive detection of organophosphorothioate pesticides such as chlorpyrifos and exhibited high selectivity and anti-interference. Importantly, it has been clearly shown that the QD chemosensor can directly detect chlorpyrifos residues at levels under the maximal residual limit in apples set by the EPA. Moreover, the construction of this fluorescent chemosensor does not involve the use of antibodies or enzymes or a complicated surface modification and thus is very simple and inexpensive. The novel and facile strategy reported herein is expected to allow application not only for the assay of organophosphorothioate pesticides in agricultural products but also for the detection of a wide range of organic and biological molecules.

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