(CdSe)ZnS Quantum Dots and Organophosphorus Hydrolase Bioconjugate as Biosensors for Detection of Paraoxon

Xiaojun Ji,[†] Jiayin Zheng,[†] Jianmin Xu,[†] Vipin K. Rastogi,[‡] Tu-Chen Cheng,[§] Joseph J. DeFrank,[‡] and Roger M. Leblanc*,[†]

University of Miami, Department of Chemistry, Coral Gables, Florida, 33124-0431, U.S. Army Edgewood Chemical & Biological Center, Aberdeen Proving Ground, Maryland 21010-542, and [3]GEO-Centers, Inc., Gunpowder Club, Aberdeen Proving Ground, Maryland 21010

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In this paper, we first report a novel biosensor for the detection of paraoxon based on (CdSe)ZnS core−shell quantum dots (QDs) and an organophosphorus hydrolase (OPH) bioconjugate. The OPH was coupled to (CdSe)ZnS core−shell QDs through electrostatic interaction between negatively charged QDs surfaces and the positively charged protein side chain and ending groups ($-NH_2$). Circular dichroism (CD) spectroscopy showed no significant change in the secondary structure of OPH after the bioconjugation, which indicates that the activity of OPH was preserved. Detectable secondary structure changes were observed by CD spectroscopy when the OPH/QDs bioconjugate was exposed to organophosphorus compounds such as paraoxon. Photoluminescence (PL) spectroscopic study showed that the PL intensity of the OPH/QDs bioconjugate was quenched in the presence of paraoxon. The overall quenching percentage as a function of paraoxon concentration matched very well with the Michaelis−Menten equation. This result indicated that the quenching of PL intensity was caused by the conformational change in the enzyme, which is confirmed by CD measurements. The detection limit of paraoxon concentration using OPH/QDs bioconjugate was about 10^{-8} M. Although increasing the OPH molar ratio in the bioconjugates will slightly increase the sensitivity of biosensor, no further increase of sensitivity was achieved when the molar ratio of OPH to QDs was greater than 20 because the surface of QDs was saturated by OPH. These properties make the OPH/QDs bioconjugate a promising biosensor for the detection of organophosphorus compounds.

1. Introduction

Semiconductor nanocrystals (or quantum dots, QDs) have attracted great interest in both fundamental research and technical applications in recent years. Because of their tunable size-dependent emission with high photoluminescence (PL) quantum yields, their broad excitation spectra, and narrow emission bandwidths, semiconductor quantum dots have been intensively investigated in versatile applications, including thin-film light-emitting devices (LEDs),[1] low-threshold lasers,[2] optical amplifier media for telecommunication networks and biological labels.[3,4] It has been proven that overcoating the quantum dots with higher band gap inorganic semiconductor materials can substantially increase the PL quantum yields and, especially, the chemical stability and photostability by passivating nonradioactive surface recombination sites.[5] Consequently, the core−shell-type QDs, such as (CdSe)ZnS QDs, have been widely used in both optoelectronic and biological applications.[6]

Organophosphorus (OP) compounds are widely used in the agriculture all over the world as pesticides and insecticides.[7] OP nerve agents used as a chemical warfare weapon are chemically related to organophosphates used as pesticide, but they are more toxic to mammals, particularly when absorbed through the skin.[8] As a matter of fact, both of them exert their toxicity through nonreversible phosphorylation and inactivation of esterase in the central nerve system.[9] The most important enzyme inhibited by OP compounds is the acetylcholinesterase (AChE). Inactivation of AChE results in the buildup of neurotransmitter, acetylcholine (ACh), and produces serious clinical complications including respiratory disorders, and fibrillation and and leads ultimately to death.[10] Because of the acute toxicity of OP compounds, the detection of them in the environment plays a very important role in keeping these compounds at a harmless level in human ecosystem. Many analytical methods have been devised to detect OP compounds, for example, gas chromatography (GC),[11] high-performance liquid chromatography (HPLC),[12] and electrical techniques.[13] These methods have proven to be sensitive and reliable but have significant disadvantages. They are time-consuming, expensive, and must be done by skilled personnel. It is believed that enzymatic biosensors can overcome those disadvantages with their high efficiency and sensitivity.

OP hydrolase (OPH) is the most well characterized enzyme that can catalyze the hydrolysis of a wide range of OP pesticides as well as nerve agents.[14] Different from AChE enzyme based biosensors, OPH-based assays respond to OP compounds as enzyme−substrates rather than inhibitors. In other words, the response of OPH-based biosensors to OP compounds can be reversible and requires only the analyte of interest. As a result of these characteristics, OPH-based enzymatic biosensors show considerable potential for development of fast-response, highly

* To whom correspondence should be addressed. Tel: (305) 284-2194. Fax: (305) 284-6367. E-mail: rml@miami.edu.
[†] University of Miami.
[‡] U.S. Army Edgewood Chemical & Biological Center.
[§] GEO-Centers, Inc.

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sensitive, and biospecific detection of a limited number of OP compounds such as paraoxon. Several types of OPH-based biosensors have been introduced recently, including potentiometric,[15] optical,[16] and amperometric[17] ones.

Hybrid systems consisting of semiconductor QDs coupled to biomaterials find growing interest in the developing research area of both biotechnology and nanotechnology. The bioconjugation of QDs yields hybrid materials, processes, and devices that can utilize both the unique optical and magnetic properties of QDs and highly selective binding to oligonucleotides and proteins.[4a,18] In particular, the attachment of proteins to QDs has recently received much attention for their applications in constructing more complex structures[19] and in new sensing and imaging technologies.[20,21]

In this research, we describe the preparation of a bioconjugate composed of water soluble (CdSe)ZnS core−shell QDs and OPH enzymes that can be used as effective OP biosensors. The trioctylphosphine oxide (TOPO)-capped (CdSe)ZnS QDs were modified by 2-mercaptoacetic acid to achieve water solubility in basic conditions with preservation of high PL quantum yield. Furthermore, the alkyl-COOH-terminated capping groups also provide (CdSe)ZnS QDs a negative surface charge distribution that can promote direct self-assembly with other molecules that have a net positive charge. As reported, this type of water-soluble QDs can form stable protein/QD bioconjugates through Coulombic interaction between the negatively charged QDs surfaces and the positively charged protein end groups.[22] The secondary structure of pure OPH, OPH/QD bioconjugates in the absence and presence of paraoxon was examined using the CD spectroscopic technique. PL intensity of the OPH/QDs bioconjugate solution was determined in a series of paraoxon solutions, and a quenching of the QDs PL was detected. The fact that the pure QDs solution shows no quenching in the presence of paraoxon leads to the conclusion that OPH plays a key role in the detection of paraoxon in our PL intensity-based sensor system. The influence of the molar ratio of OPH in the bioconjugate to the sensitivity of biosensor was also investigated in this work.

2. Experimental Section

2.1. Materials and Methods. Cadmium oxide (CdO), selenium (Se), TOPO, trioctylphosphine (TOP), hexamethyldisilathiane [$(TMS)_2S$], 2-mercaptoacetic acid, and paraoxon (diethyl-*p*-nitrophenyl phosphate) were purchased from Sigma-Aldrich (Milwaukee, Wisconsin). The tetradecylphosphonic acid (TDPA) was obtained from Alfa Aesar (Ward Hill, Massachusetts). The diethylzinc ($ZnEt_2$, 15 wt % solution in hexane) was obtained from Acros Organics (Morris Plains, New Jersey). All the starting materials for the synthesis and surface modification of (CdSe)ZnS core−shell QDs were directly used without any further purification. OPH (75%) (E. C.3.1.8.1) was isolated, extracted, and purified by the U.S. Army Laboratory (Edgwood Chemical and Biological Center, Maryland). A stock solution of OPH (1.5 mg/mL) was prepared in 100 mM bis-tris-propane (BTP) buffer, pH = 7.3, containing 10 μM Co^{2+}. The stock solution was frozen and stored at −4 °C. The stock solution of paraoxon was prepared by dissolving the paraoxon in 50% (v/v) CH_3OH/H_2O mixture to the concentration of 4.63×10^{-3} M and stored at 4 °C. The stock solution of paraoxon was further diluted with 50% (v/v) CH_3OH/H_2O to correspondent concentrations.

2.2. Synthesis of (CdSe)ZnS Core−Shell QDs. Briefly, the (CdSe)ZnS core−shell QDs capped with a TOPO ligand were prepared through a stepwise procedure as described elsewhere.[23] The precursor of (CdSe)ZnS core−shell QDs, nearly monodispersed TOPO-coated CdSe QDs, were synthesized by the method reported previously by Peng and Peng.[24] It our case, the cadmium oxide (CdO) and tetradecylphosphonic acid (TDPA) were used as Cd precursor instead of the organometallic reagent $Cd(CH_3)_2$ and trioctylphosphine selenide as Se precursor. The CdSe QDs were formed by pyrolysis of Cd and Se precursors in a coordinating solvent, TOPO, at high temperature (∼250−270 °C). The QDs were collected as powders using size-selective precipitation[25] with methanol and dried in a vacuum. The average size of CdSe/TOPO QDs were determined by UV−vis absorption spectroscopy of CdSe/TOPO in chloroform solution.[26] Approximate particle concentration of CdSe/TOPO QDs solution can also be determined via UV−vis measurements.[26] Thus, by knowing the mass of CdSe/TOPO QDs used for preparing the solution and the total volume of the stock solution, the "molecular" weight of CdSe/TOPO QDs can be estimated and used in further synthesis.

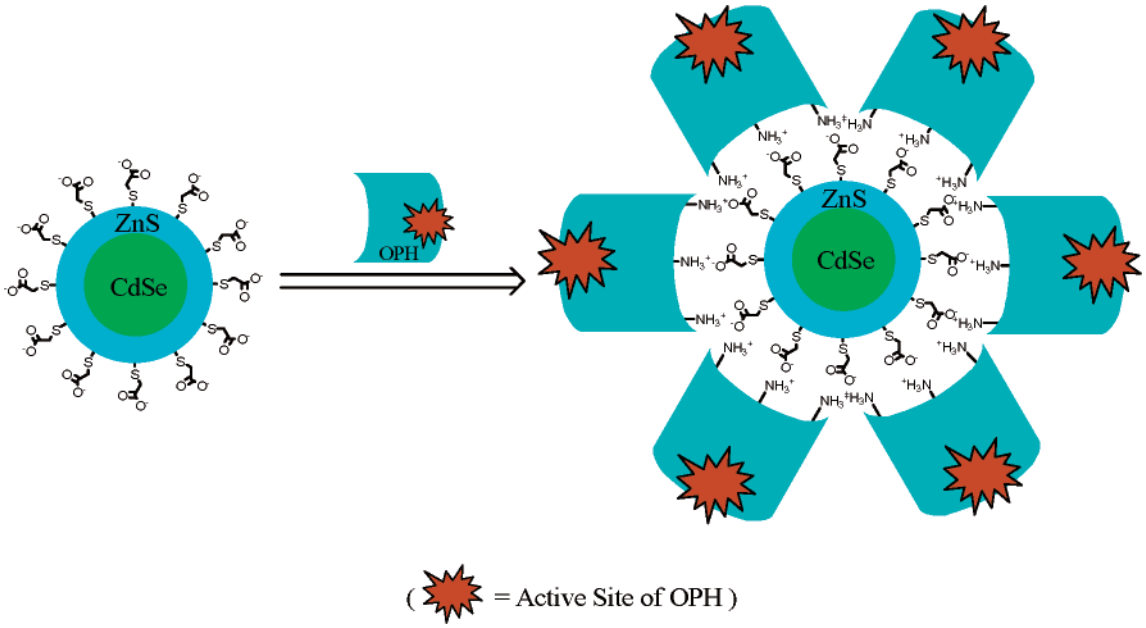
$ZnEt_2$ and $[(TMS)_2S]$ were used as Zn and S precursors for the ZnS cap. The amount of Zn and S precursors needed to grow a ZnS shell of desired thickness for each CdSe sample was determined as follows: first, the average size of CdSe QDs was estimated from UV−vis measurements. Next, the mole number of ZnS necessary to form a shell per mole of CdSe QDs was calculated based on the volume of individual CdSe QDs and the desired thickness of the shell. A spherical core and shell shape were assumed, and the bulk density of ZnS was used in the calculation. Then, the amount of ZnS for one synthesis was calculated by knowing the mass of CdSe/TOPO QDs used and the "molecular" weight of CdSe/TOPO QDs estimated previously from UV−vis measurements. Finally, the amount of $ZnEt_2$ and $(TMS)_2S$ was calculated from the amount of ZnS needed in this synthesis. For example, if the CdSe QDs has an average size of 2.48 nm and a "molecular" weight of 32 000, the amount of ZnS needed for a 1-nm shell for each single particle will be 1.599×10^{-19} g. Thus, by knowing the amount of CdSe QDs, the amount of $ZnEt_2$ and $(TMS)_2S$ can be calculated.

The common procedure of coating was carried out by adding the $ZnEt_2$ and $(TMS)_2S$ mixture solution dropwise into a high-temperature coordination solution (TOPO/TOP as solvent) of CdSe QDs.[23] Usually, the temperature of the coating procedure was lower than that used during CdSe nanocrystals growth to avoid compromising the integrity of the native cores. The (CdSe)ZnS core−shell QDs were collected as powder by precipitation and washing with methanol followed by drying in a vacuum.

2.3. Modification of (CdSe)ZnS/TOPO QDs. Surface−ligand exchange of (CdSe)ZnS/TOPO QDs with 2-mercaptoacetic acid made the QDs water soluble in basic aqueous solution. The procedure of the surface−ligand exchange has been reported elsewhere.[18a] (CdSe)ZnS/TOPO QD powder was dissolved in chloroform, an excess amount of 2-mercaptoacetic acid was added into the QDs solution, and an instant formation of precipitate can be observed. The precipitate was collected via centrifugation after 2 h of stirring, washing 3 times with chloroform, and drying in a vacuum. The dried QDs were then suspended in 0.1 M NaOH solution to get a clear solution. Any insoluble substances were removed by centrifugation. The concentration of the QDs aqueous solution can be determined by the same method used for determination of the QDs chloroform solution described above.

2.4. Bioconjugation of QDs with OPH. As described previously, the (CdSe)ZnS QDs and proteins can form stable

SCHEME 1: Proposed Scheme for the Formation of OPH/QDs Bioconjugates



protein/QD bioconjugates through a self-assembly strategy.[22,27] Because the isoelectronic point of OPH is 7.6, the OPH molecule is partially positively charged at pH = 7.3. This makes it possible to be attached to the negatively charged QDs surface. Bioconjugate complexes were prepared by simply mixing the QDs and OPH in PBS buffer (pH = 7.3) solution at room temperature (Scheme 1). For example, approximately 20 μL of OPH solution (10^{-5} M, 100 mM BTP buffer, pH = 7.3, Co^{2+} = 10 μM) was added into 200 μL of QD solution (10^{-6} M, pH = 12.5) and allowed to self-assemble for ~15 min at room temperature. Molar ratios of OPH to QDs were discretely varied among samples from 10 to 200 in order to investigate the concentration effect of OPH on the sensitivity of the biosensor. In the case of a molar ratio of 200, a more concentrated OPH stock solution (2×10^{-5} M) was used to keep the overall QDs concentration the same as the other samples. The individual samples were then diluted with BTP buffer to a total volume of 4 mL. Usually, the QDs aqueous solution is quite stable at high pH values (e.g., pH = 12), while the decrease in pH value (e.g., pH = 6) of QDs solution always results in aggregation. Although the QDs solution we used will precipitate eventually (less than 1 day) at pH < 8, even at very low concentration (10^{-8} M), the OPH/QD bioconjugate BTP buffer solutions (pH = 7.3) were free from aggregation for at least 1 week. This could be indirect evidence for the formation of OPH/QD bioconjugates. In addition, the reference QDs-only solution was also prepared by diluting 200 μL of QDs stock solution (10^{-6} M, pH = 12.5) with BTP (100 mM BTP, pH = 7.6, Co^{2+} = 10 μM) to a total volume of 3 mL. Because of the aggregation problem of QDs in nonbasic medium, the reference QDs-only solution was prepared immediately before use.

2.5. Circular Dichroism (CD) Measurements of OPH and OPH/QDs Bioconjugate. CD is one of the most sensitive physical techniques for determining structures and monitoring structural changes of biomolecules. It can directly interpret the changes of protein secondary structure.[28] The most utilized form of CD spectroscopy is far-UV CD (190−260 nm). This corresponds to electronic transitions of the backbone chromophore of the enzyme.[29] Recently, CD has been widely used to analyze the main properties and features of enzyme solutions and films,[30] which show a bright future for this spectroscopic technique in biosensor research.

The OPH/QDs bioconjugate stock solution (OPH concentration = 1×10^{-6} M, OPH/QDs = 10:1 molar ratio) was prepared and used for the preparation of CD samples. The reference solution with only OPH was prepared by simply diluting the OPH stock solution with BTP buffer while keeping the same OPH concentration as in the OPH/QDs bioconjugate stock solution. All samples were then prepared by diluting 500 μL of stock solutions with paraoxon solution (4.67×10^{-6} M in 50% CH_3OH/H_2O (v/v)) and 50% CH_3OH/H_2O (v/v) to a total volume of 1 mL. By use of this method, we were able to keep each sample at the same OPH concentration.

All the CD spectra were taken using a Jasco J-810 spectropolarimeter fitted with a 150-W xenon lamp. Quartz cells of 1-mm optical path length were used for all CD measurements of OPH and OPH/QDs solutions, and the spectra were recorded in the far-UV region (190−260 nm) with a response time of 8-s intervals and scan speed of 50 nm/min. Three scans were accumulated and averaged for each spectrum after the background of the buffer solution was subtracted.

There are three popular methods (SLECON3, CONTIN, CDSSTR) for estimating protein secondary structure fractions from CD spectra.[31,] But it is difficult to compare the results from different methods because of the different reference sets and different secondary structure assignments used in these methods of analysis.[32] CDPro software package consists of these three programs and a program for determining tertiary structure class (CLUSTER). One of the major advantages for the CDPro software package is that the programs have been modified to accept any given set of reference proteins (CD spectra and secondary structure fractions), and seven such reference sets are provided. Moreover, input data files for these three programs are identical. More information about CDPro is available at the website http://lamar.colostate.edu/~sreeram/CDPro. The assignment from CDPro gave six secondary structural classes: regular α-helix, α_R; distorted α-helix, α_D; regular β-strand, β_R; distorted β-strand, β_D; turns, T; and unordered, U. In this paper, we used the CDPro software package to estimate the average secondary structure fraction in OPH and OPH/QD bioconjugate solutions in the absence and presence of paraoxon.

TABLE 1: Secondary Structure Data of OPH, OPH/QDs Bioconjugates, and OPH/QDs Bioconjugates in 10^{-6} M of Paraoxon[a]

	percentage of secondary structure					
	α-helix		β-strand			
	α_R	α_D	β_R	β_D	turn	unorder ed
OPH	40.03	25.53	2.40	4.43	13.67	15.53
OPH/QDs bioconjugates	36.10	21.43	3.37	4.10	13.10	20.07
OPH/QDs bioconjugates in the presence of paraoxon (10^{-6} M)	30.33	30.30	17.33	7.90	22.33	8.13

[a] The subscripts "R" and "D" represent "ordered" and "disordered", respectively.

2.6. Photophysical Measurements. Several sets of samples were prepared by diluting 300 μL of OPH/QDs bioconjugate stock solutions (OPH:QDs = 10:1 and 100:1, molar ratio) with paraoxon stock solutions (4.67×10^{-5} and 4.67×10^{-6} M, 50% CH_3OH/H_2O (v/v) as solvent) and 50% CH_3OH/H_2O (v/v) solvent to a total volume of 3 mL, and the final concentration of paraoxon was then calculated. The reference samples were prepared by diluting 300 μL of reference QDs-only solution to a total volume of 3 mL with 50% CH_3OH/H_2O (v/v) solvent.

The samples for the fluorescence measurements were then placed in a 10-mm optical path length quartz fluorescence cuvette, and the emission spectrum of each sample was measured using a SPEX fluoroMax spectrofluorometer (SPEX Industries, Edison, NJ). All samples were excited at 350 nm.

3. Results and Discussion

3.1. Synthesis and Modification of (CdSe)/ZnS Core−Shell QDs. The TOPO-capped (CdSe)ZnS QDs can be suspended in many nonpolar solvents such as chloroform, dichloromethane, and hexane. The resulting (CdSe)ZnS core−shell QDs were rendered water soluble by replacing the native TOPO organic capping with 2-mercaptoacetic acid ligands. The 2-mercaptoacetic acid capped QDs have a homogeneous density of negative charge due to deprotonation of the carboxylic acid end groups. Consequently, the QDs stock solutions were exceptionally stable in basic pH buffer. The 2-mercaptoacetic acid capped QDs samples were stable over a period of several months and have been routinely prepared in our laboratory and used for various applications.

3.2. CD Spectroscopic Study of OPH/QD Bioconjugates. As described above, the OPH/QDs bioconjugate is formed through self-assembly, electrostatic interaction between the negatively charged QDs surface, and the positively charged protein ending groups. Figure 1 shows the CD spectra of OPH and OPH/QD bioconjugates in the absence and presence of paraoxon. Before the bioconjugation, the spectra showed two strong double minima at 222 and 209 nm and a stronger maximum at 194 nm, which is typical of high α-helical content.[28] The crossover point at 201 nm was also assigned to the α-helix structure. The 222-nm peak has a larger intensity than the 209-nm peak, showing that the 75% OPH enzyme is more like an α/β type.[28] Table 1 shows the average fraction of the secondary structure estimated from the CD data using the CDPro software package. Before the conjugation, the regular and distorted α-helix contents were 40.03 and 25.53%, respectively. After the conjugation with QDs, the CD spectrum looked similar, but the difference between the two minima became less obvious. This is confirmed by the calculated values from CDPro, which indicated a decrease in the percentage of both regular and distorted α-helices. The positive peak only shifted slightly from 194 to 192 nm, indicating that there was no significant change in the secondary structure of the enzyme. When paraoxon was added into the OPH/QDs bioconjugate, there was not much change in the CD spectrum, except the positive peak

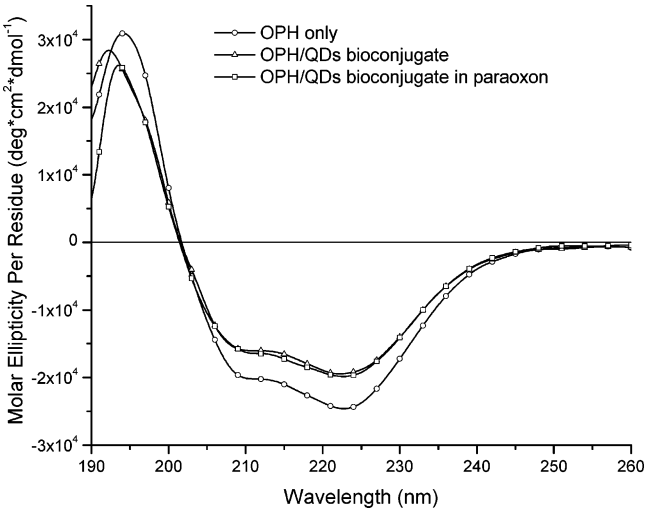


Figure 1. CD spectra of pure OPH and OPH/QDs bioconjugates with and without paraoxon. OPH concentration is 5×10^{-7} M in all cases. The OPH/QDs molar ratio of bioconjugate is 10. Paraoxon concentration is 10^{-6} M.

which shifted from 192 to 193 nm. The CDPro data showed that the percentage of β-strand increased from 3.37 to 17.33% in the presence of paraoxon, which may result from the binding and hydrolysis of paraoxon. We cannot see this change in the CD spectrum because the intensities of CD spectrum related to α-helix usually predominate those of β-strand for α/β enzymes.[28]

Although a slight change of secondary structure of OPH occurred during the formation of bioconjugate according to the CD spectroscopic data, the following PL study of OPH/QD biosensors indicated that this small change did not inhibit the enzymatic activity of OPH. Similar phenomenon (i.e., the preservation of catalyst activity of enzyme after bioconjugation) was also found and reported by Zhang et al.[33] when they conjugated the protein trichosanthin to the surface of core/shell QDs.

3.3. PL Spectroscopic Studies of the OPH/QD Bioconjugates. The PL properties of the OPH/QDs bioconjugates solutions were utilized to detect trace amounts of paraoxon in aqueous solution. The (CdSe)ZnS QDs showed a strong PL at 570 nm, which corresponds to a CdSe core size of 3.1 nm with a narrow fwhm (full width at half maximum) of 30 nm.

The PL spectra of the OPH/QDs bioconjugates were measured in paraoxon solutions at different concentrations (parts a and b of Figure 2). An observable decrease of PL peak intensity was found both in 10:1 and 100:1 (molar ratio) bioconjugates using an excitation at 350 nm. The PL intensity of QDs decreased as the concentration of paraoxon increased. Both systems showed the highest detection at about 4.2×10^{-8} M of paraoxon. In the case of pure QDs samples, no quenching was observed at the range of concentrations of paraoxon investigated.

On the basis of the fact that paraoxon did not show detectable quenching effect of the QDs (Figure 2c), we believe that the change of the secondary structure of OPH may account for the

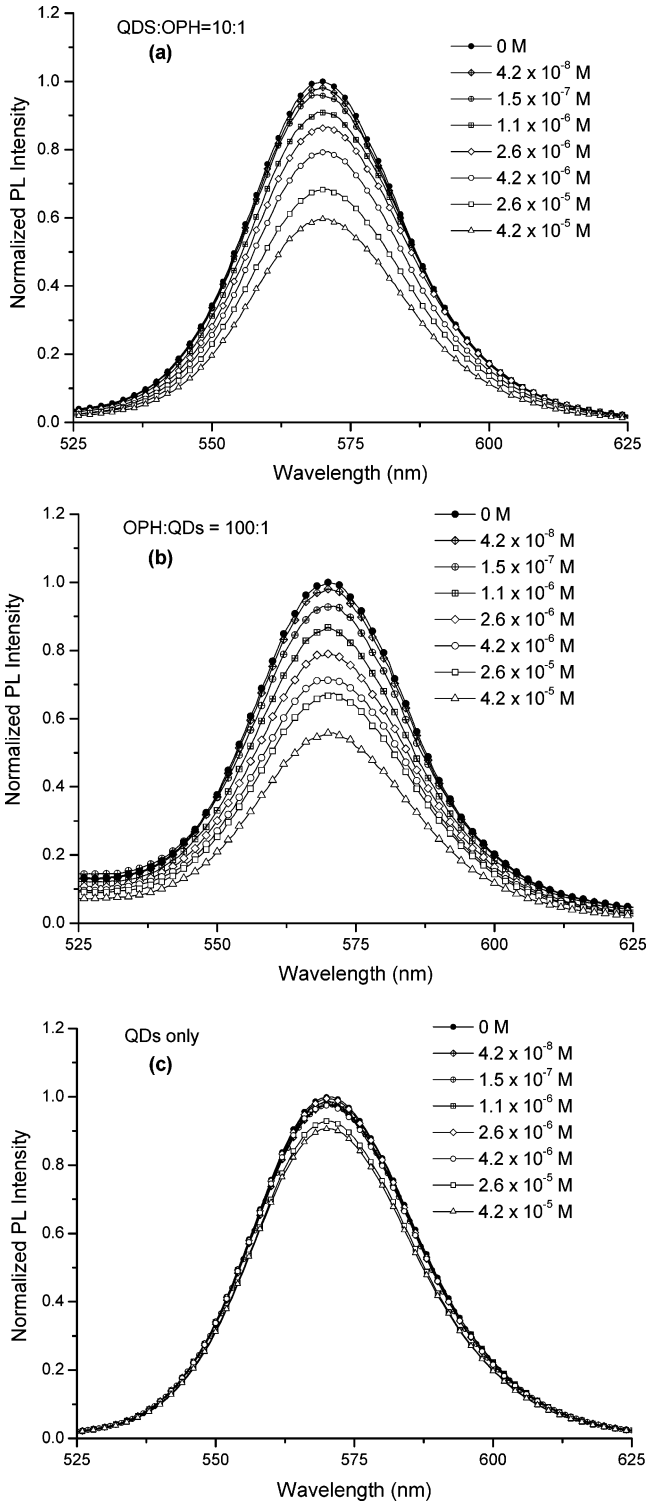


Figure 2. PL spectra of (a) 10:1 and (b) 100:1 molar ratio OPH/QDs bioconjugate; (c) pure QDs in different concentrations of paraoxon. All samples were excited at 350 nm.

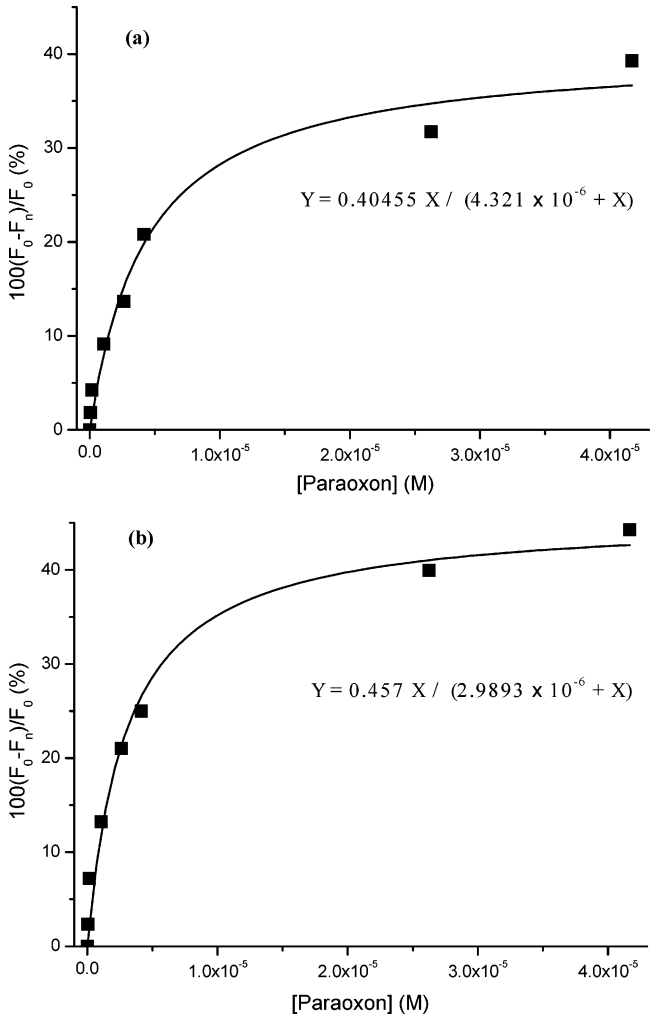
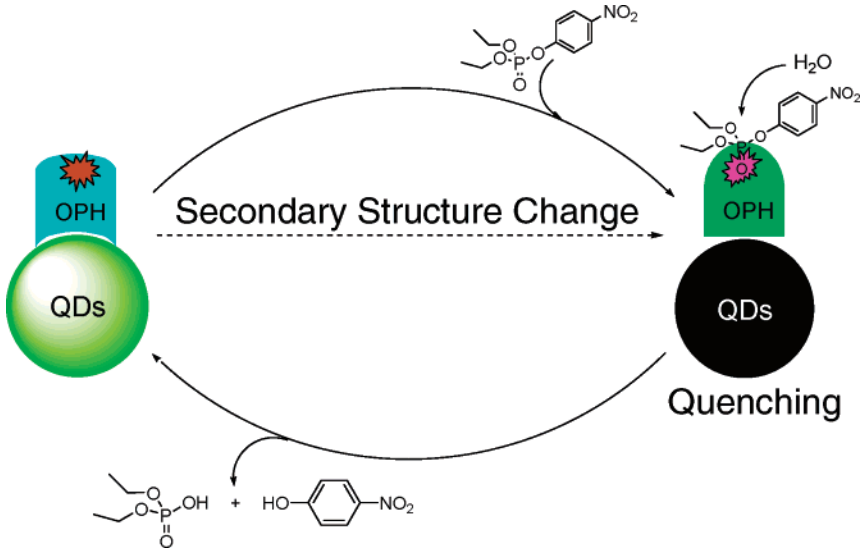


Figure 3. Relative PL intensity percentage of (a) 10:1 (molar ratio) and (b) 100:1 (molar ratio) OPH/QDs bioconjugates as a function of paraoxon concentration. F_0 and F_n represent the PL intensity of bioconjugates at emission maximum in the absence (F_0) and presence (F_n) of paraxon. The equations of the hyperbolic fit of two data sets are given in the graph.

quenching of the PL intensity of the QDs by influencing the degree of surface passivation.[20] This binding process ultimately leads to quenching of the PL intensity of QDs. (Scheme 2) This assumption can be proven by an enzymatic kinetics study of OPH. As discussed before, the OP compound interacts with OPH as a substrate rather than an inhibitor. This property leads to a direct determination of the concentration of the analyte as the rate of signal generation. On the basis of Michaelis' and Menten's theory,[34] the concentration of enzyme−substrate complex which is proportional to the reaction rate has a hyperbolic dependence on the initial concentration of substrate and remains constant during the reaction. If we plot the relative PL intensity percentage against paraoxon concentration, a hyperbolic dependence can be found in both 10:1 and 100:1 bioconjugates (Figure 3). The hyperbolic fit of two sets of data gave the maximum quenching percentages at 40.4 and 45.7% for 10:1 and 100:1 (molar ratio) bioconjugates, respectively, which implied that the catalytic reaction has a maximum reaction rate due to the saturation of active sites on the enzyme. This fact leads to the conclusion that the quenching percentage is directly proportional to the concentration of enzyme−substrate complex. In other words, the secondary structure change of OPH due to the binding of paraoxon or the formation of enzyme−substrate complex is responsible for the quenching of the intensity of PL.

As a matter of fact, the OPH/QDs bioconjugate works not only to detect the presence of paraoxon in solution but also to detoxify the paraoxon by catalyzing the hydrolysis reaction. It may be possible that the hydrolysis product *p*-nitrophenol (PNP) can affect the PL intensity of QDs. To address this question, the PL spectra of QDs-only solution in series of PNP solutions were taken and showed no significant change in the intensity of PL. (Data not presented.)

SCHEME 2: Proposed Mechanism of the Quenching of PL Intensity



3.4. Concentration Effect of OPH to QDs to the Sensitivity of Biosensor. On the basis of the Michaelis−Menten curves of the two OPH/QDs bioconjugates (Figure 3), the bioconjugate of 100:1 has a larger maximum quenching percentage (45.7%) compared to the 10:1 (40.5%). That means that the sensitivity of the OPH/QDs biosensor increased slightly with the increase of OPH to QDs molar ratio. Is it not the way to improve the sensitivity of the biosensor? To answer this question, different samples with various OPH/QDs molar ratios were prepared and the intensity of PL were measured for two concentrations of paraoxon, see Figure 4. The degree of quenching of the intensity of PL by paraoxon increased very little when the molar ratio of OPH was increased. And no further increase of sensitivity can be achieved when the OPH/QDs ratio is greater than 20. We explain these results in the following way. The (CdSe)ZnS QDs has a 3.1-nm diameter CdSe core and about a 1-nm ZnS shell. If the mercaptoacetic acid capping was counted, the average size of QDs is almost the same size as an OPH molecule ($d \approx$ 55 Å).[35] If a spherical shape for both species and a close-packed coordination were assumed, there should be at most 12 OPH molecules bonded to the surface of each individual QDs. Increasing the molar ratio of OPH will only result in the increase of unbonded OPH after the surface coverage of QDs is saturated by OPH molecules. Because the conformational change of OPH affected the intensity of PL by altering the degree of surface passivation of QDs, those free OPHs that are not attached to the surface of QDs will have little effect on the PL properties of QDs.

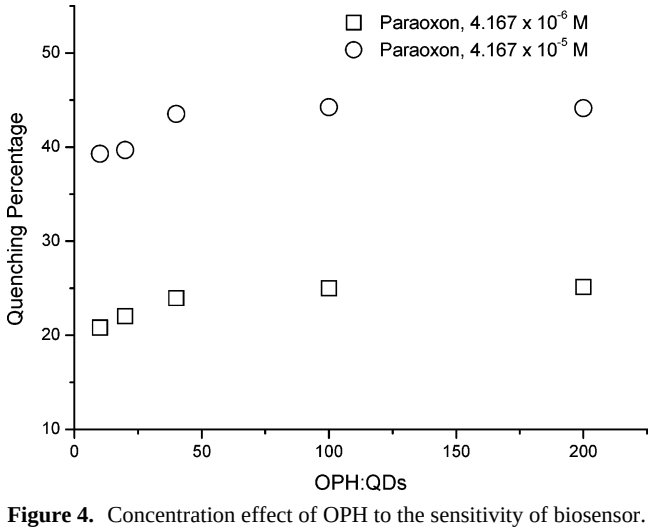


Figure 4. Concentration effect of OPH to the sensitivity of biosensor.

4. Conclusion

In this study, a novel OPH/QDs bioconjugate-based biosensor is reported. The bioconjugate was prepared through self-assembly to detect trace amount of paraoxon in solution. The CD spectra of OPH/QDs bioconjugates in free bioconjugate solution and in the presence of paraoxon solution provide a proof of secondary structural change of OPH, which is induced by the binding and hydrolysis of paraoxon. PL spectroscopy revealed that the PL intensity of QDs decreased when the concentration of paraoxon in aqueous solution increased. This quenching of PL intensity can be used as an effective biosensor with a detection limit of 10^{-8} M of paraoxon. It is assumed that the quenching of PL intensity results from the conformational change of OPH induced by the paraoxon substrate. The hyperbolic dependence of the relative PL intensity on the concentration of paraoxon proved that assumption. Furthermore, increasing the amount of OPH in the bioconjugate will not substantially increase the sensitivity of the biosensor because of the surface saturation of QDs by OPH. The OPH/QDs bioconjugate-based biosensor represents a novel and promising type of enzymatic biosensor for the detection of paraoxon. The results from CD and PL spectroscopies in this paper are also of great value for research on the interaction between proteins and QDs.

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