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Time-Dependent Photoluminescence Blue Shift of the Quantum Dots in Living Cells: Effect of Oxidation by Singlet Oxygen

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Abstract: Time-dependent photoluminescence (PL) enhancement, blue shift, and photobleach were observed from the thiol-capped CdTe quantum dots (QDs) ingested in mouse myoblast cells and human primary liver cancer cells. It was revealed that the PL blue shift resulted from the photooxidation of the QD core by singlet oxygen molecules formed on the QD core surface.

Introduction

Fluorescence labeling is one of the most commonly used techniques in cell biology.[1,2] Comparing with the traditional cellular labeling fluorophores, semiconductor quantum dots (QDs) have the advantages of higher photoluminescence (PL) efficiency, reduced tendency to photobleach, and narrow emissions with a broad excitation band. The nanosize of QDs gives them unique optical properties, for example, their emissions can be precisely tuned from the UV to the IR region only by changing their size, which makes them possible for multiplex labeling.[3] The first study of using QDs for cellular labeling was reported by Chan and Nie in 1998.[4] In recent years, along with the development of QDs, there has been increasing interest in their potential applications in labeling of cells, protein trafficking, DNA detection, etc.[5−10] For those applications, water-soluble and highly luminescent II−VI QDs, such as colloidal CdSe and CdTe QDs, are mostly adopted, and their physical and chemical properties have been widely studied.[3,11] When studying the optical properties of the colloidal QDs, investigations have been focused on their PL behaviors. Phenomena such as photoenhanced luminescence, photoinduced spectral blue shift, and on/off behavior (blinking) have been observed in QD solutions as well as for single QDs.[12−15] Subsequently, many theories have been developed to explain these phenomena.[16−18] For biological and medical applications, it is of particular importance to study the photophysical properties of QDs in living cells. However, few works concerning the PL behavior of QDs in living cells have been reported so far. The photoinduced optical properties of the intracellular QDs are far from being fully understood. In this work, the PL spectroscopy of the thiol-capped CdTe QDs in living cells was studied. Time-dependent PL enhancement, spectral blue shift, and photobleach of the intracellular QDs were observed, and the mechanism of the photoactivation process was explored.

Experimental Section

The water-soluble thiol-capped colloidal CdTe QDs with an average core diameter of about 3.5 nm were synthesized using a previously reported method.[19] Briefly, by a molar ratio of 2:1, sodium borohydride was used to react with tellurium in water to prepare sodium hydrogen telluride (NaHTe). Fresh solutions of NaHTe were diluted with N_2-saturated deionic water to 0.0467 M for further use. $CdCl_2$ (1 mmol) and thioglycolic acid (1.2 mmol) were dissolved in 50 mL of deionized

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water. Stepwise addition of NaOH solution adjusted the precursors solution to pH = 9. Then, 0.096 mL of oxygen-free solution containing fresh NaHTe, cooled to 0 °C, was added into 10 mL of the above precursor solution and vigorously stirred. Finally, the solution with a faint yellow color was put into a Teflon-lined stainless steel autoclave with a volume of 15 mL. The autoclave was maintained at the reaction temperature (200 °C) for a certain time and then cooled to room temperature by a hydrocooling process.

The uptake of QDs into living cells was carried out as follows: The mouse myoblast cells (C2C12) or human primary liver cancer cells (PLC) procured from the Cell Bank of Shanghai Science Academy were seeded onto a glass cover slip placed in a culture dish containing DMEM-H medium with 10% calf serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 100 μg/mL neomycin. The cells were then cultured in a fully humidified incubator at 37 °C with 5% CO_2. When the cells adhered to the cover slip and reached 80% confluence with normal morphology, the CdTe QD aqueous solution was added into the culture dish to achieve a QD concentration of 100 μg/mL. These cells were then incubated for 4 h in an incubator. After incubation, the cover slip with the adhered living cells was washed with PBS (phosphate-buffered saline) three times to remove the unbound QDs and then sealed on a glass slide for microscopic measurement. With such a QD concentration in solution, no detectable damage to cells was observed.

The intracellular QD distributions were detected with a laser scanning confocal microscope (Olympus, FV-300, IX71) using a 405 nm semiconductor laser (Coherent) as the excitation source. The laser beam was focused by a 60× objective to a spot of about 1 μm in diameter. On the basis of the obtained micrographs, we selected some parts in the cells to measure the micro-PL spectra of the intracellular QDs using the point-stay mode of the microscope. The excitation power density used was about 250 W/cm^2. The spectra were measured using a spectrometer (Acton, spectropro 2150i) equipped with a liquid-nitrogen-cooled CCD (Princeton, Spec-10:100B LN). The PL output from the side exit of the microscope was directly focused onto the entrance slit of the spectrometer.

Results

The QD distributions in C2C12 and PLC cells are depicted in Figures 1 and 2, respectively. Using the *z*-scan mode, the images in different layers were recorded to detect the distributions of QDs inside the cells. From those images the three-dimensional intracellular distribution of QDs was obtained. In Figures 1 and 2, A shows the fluorescence micrographs recorded with a 585−640 nm band-pass filter, where the main image exhibits the QD distribution in an $X-Y$ plane inside the cell and the images displayed below and at the right side of the main one are the $X-Z$ and $Y-Z$ profiles obtained by *z*-scanning along the marked lines in the main image, showing the cross sections of the cell. B shows the differential interference contrast (DIC) micrograph to exhibit the cell morphology, which was acquired in a transmission channel simultaneously. C shows the merged image of A and B.

The QDs were found to distribute diffusely inside the cells according to the three-dimensional micrographs (Figures 1A and 2A), indicating that the uptake of QDs into the living cells really occurred.

The PL spectra of the intracellular QDs with different irradiation times are demonstrated in Figure 3A and 3B for C2C12 and PLC cells, respectively. As an ensemble luminescence from the QD aggregates, the spectra are relatively broader as compared with that from single dot.[13,18] In both kinds of cells the spectra recorded at later times were clearly blue shifted with respect to the initial wavelength of 602 nm (peak wavelength). The PL intensity was slightly enhanced first (within 10 s) and then decreased with time. A blue shift of about 35 nm was observed 15 min before the emission was fully photobleached.

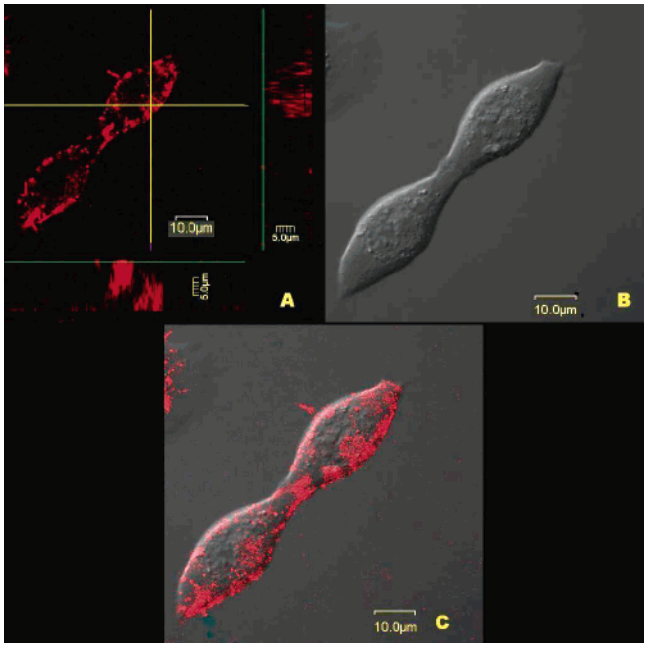

Figure 1. Micrographs of a QD-labeled C2C12 cell. (A) Micrographs of QD distributions. The main image exhibits a QD distribution in the $X-Y$ plane inside the cell. The images displayed below and on the right side of the main one are the $X-Z$ and $Y-Z$ profiles measured by *z*-scanning along the marked lines in the main image. (B) DIC micrograph. (C) Merged image of A and B.

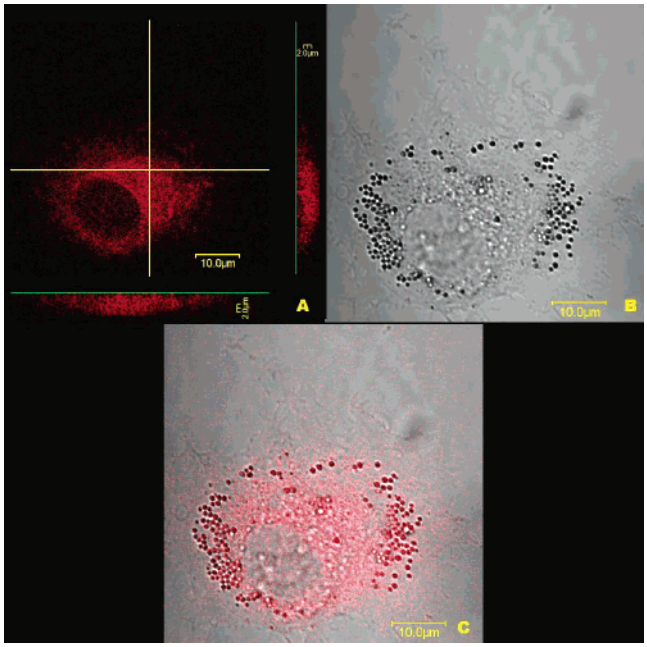

Figure 2. Micrographs of a QD-labeled PLC cell. (A−C) Images as described in Figure 1.

The PL blue shift and photobleach of QDs were previously observed in QD solutions as well as for single QDs and believed to be attributed to the irreversible photooxidation of QDs.[13,18,20,21] As a result of photooxidation, macroscopic oxide layers were

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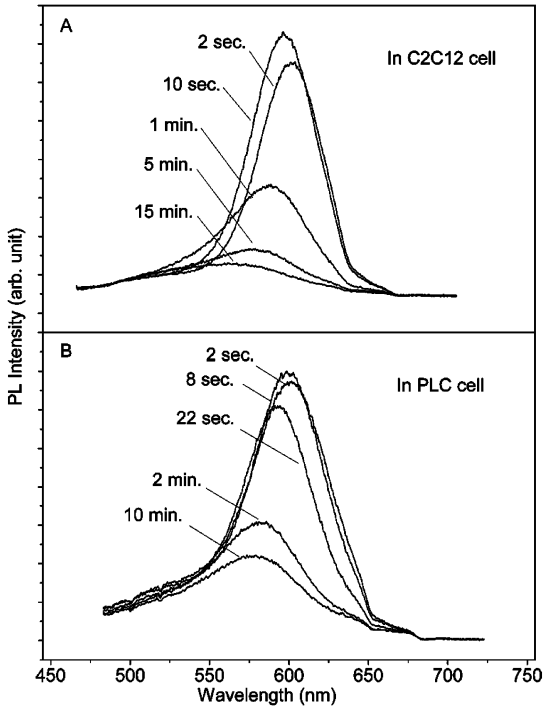

Figure 3. Time evolutions of the PL spectra of CdTe QDs in a C2C12 cell (A) and PLC cell (B).

created and the effective QD size was reduced with time. The quenching states were expected to be formed at the interface between CdTe and the surface oxide,[18] and new surface defects were also possible to be generated by oxidation of the QD surface. Hence, the PL intensity decreased and finally the QDs were bleached.[22] On the basis of the well-known quantum confinement effect,[23,24] the observed blue shift of approximately 35 nm in this work corresponds to a shrinkage in the diameter of about 0.33 nm.

PL enhancement of colloidal QDs was observed previously in pure QDs,[25] aqueous and nonaqueous solutions,[12,21] QD films between insulating layers,[26] and QD monolayers.[22] Various mechanisms were proposed to explain this effect.[12,20−22,27] Regardless of different models with complex kinetics presented, the primary reason for photoenhancement is believed to be passivation of the core surface. Passivation of the surface states that are not blocked by the capping agent causes PL enhancement, which does not change the shape and position of the PL peak.[20,21] Although the precise nature of the passivation process is not yet fully understood, with the effects of passivation and photooxidation, the time evolutions of the PL spectra observed in this work can be well explained. Passivation of surface traps enhances luminescence, and oxidation of the QD cores reduces the QD size and introduces quenching states and new defects,

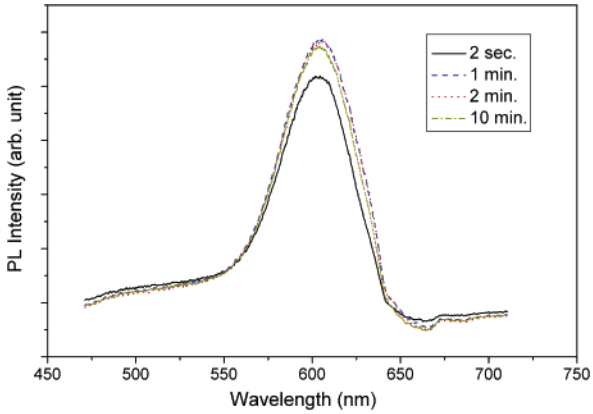

Figure 4. Time evolution of the PL spectra of CdTe QDs in a C2C12 cell deoxygenated by purging with nitrogen.

leading to the blue shift and photobleach. It is the competition between the two processes that is responsible for the complex kinetics of the luminescence observed.[20,22]

Although the time-dependent PL behavior observed in living cells can be explained by photooxidation, it is necessary to further confirm this effect directly by experiments. Therefore, the QD-loaded C2C12 cells were bubbled with nitrogen gas for 30 min to purge away oxygen molecules. The samples for microscopic measurement were then prepared in a glovebox filled with flowing pure nitrogen gas. A time evolution of the PL spectra from a deoxygenated C2C12 cell is shown in Figure 4. Contrary to the situation without deoxygenation (as shown in Figure 3A), all spectral peaks were almost at the same wavelength. No significant shift was detected. The PL intensity was enhanced slightly at the very beginning and then kept constant. These results provide unambiguous evidence that photooxidation played a dominant role in the spectral blue shift and luminescence quenching. The photooxidation is likely a universal effect for single QDs,[13,18] the monodisperse QDs in solutions,[21] or aggregate QDs in living cells. Removing oxygen molecules can therefore prevent photooxidation of QDs effectively.

However, photoenhancement of PL in C2C12 cells was observed at the beginning of the illumination in either air or a nitrogen atmosphere (Figures 3 and 4). Similar results were reported in QD aqueous solutions by Wang et al.[21] They suggested that the slight PL enhancement in N_2 was attributed to the presence of a residual amount of oxygen.

To eliminate the influence of the complex environment in cells, PL spectroscopy of QDs was studied in a deoxygenated aqueous solution. A QD aqueous solution (1 mg/mL) was bubbled with nitrogen gas for 30 min to purge away oxygen and then sealed between two cover slips in a nitrogen atmosphere. The PL spectra of this prepared sample were also measured with the confocal microscope but at a higher power density of 3.7 kW/cm^2. The PL spectra as a time evolution are shown in Figure 5. As expected, no significant enhancement, blue shift, or photobleach was observed. On the contrary, without purging with nitrogen (as can be seen later in Figure 12A), the PL spectra from the QD aqueous solution were remarkably blue shifted and photobleached with irradiation time. These results strongly support our above explanations.

To identify the nature of the products formed by photooxidation, the QD powders extracted from the solutions before and

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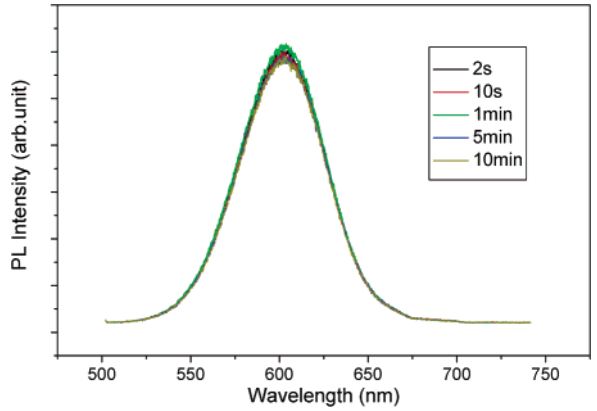

Figure 5. Time evolution of the PL spectra of CdTe QDs in aqueous solution deoxygenated by purging with nitrogen.

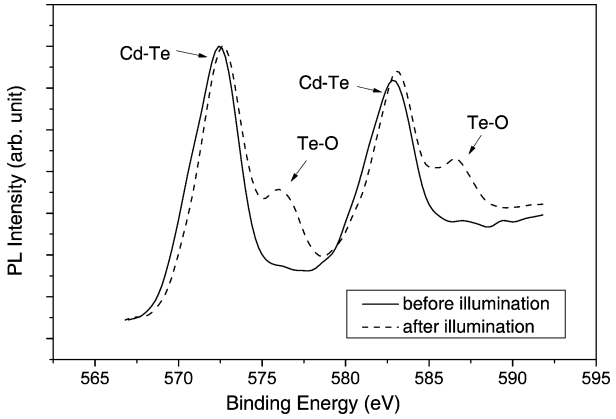

Figure 6. High-resolution XPS spectra of Te $3d_{5/2}$ and $3d_{3/2}$ peaks from CdTe QDs before (solid line) and after (dashed line) laser irradiation.

after laser irradiation were analyzed by X-ray photoelectron spectroscopy (XPS). The solutions were dripped on silicon wafers and dried in a vacuum chamber. The dried samples were carefully kept in a nitrogen atmosphere until XPS measurements were conducted. The measured high-resolution XPS spectra of the Te $3d_{5/2}$ and $3d_{3/2}$ peaks before and after laser irradiation are shown in Figure 6. It can be seen clearly in the figure that after laser irradiation two additional peaks appeared at binding energies of 576.0 and 586.4 eV, corresponding to the Te−O bonding states in $CdTeO_3$.[28] This information further supports the above conclusion that photooxidation reaction occurred during laser irradiation. Since the difference of the binding energies between the Cd 3d peaks of CdTe and CdO is only 0.1 eV,[28] with the limitation of the XPS resolution, this small chemical shift cannot give an explicit conclusion about formation of CdO although we have in fact detected a 0.2 eV shift to lower energy. However, since CdO and SeO_2 were proposed to be produced in the oxidation reactions of CdSe QDs,[29−31] CdO and TeO_2 are therefore also possible products from the oxidation reactions of CdTe QDs.

To gain further insight into the photooxidation kinetics, additional experiments were conducted with histidine, sodium azide (NaN_3), and deuterated water.

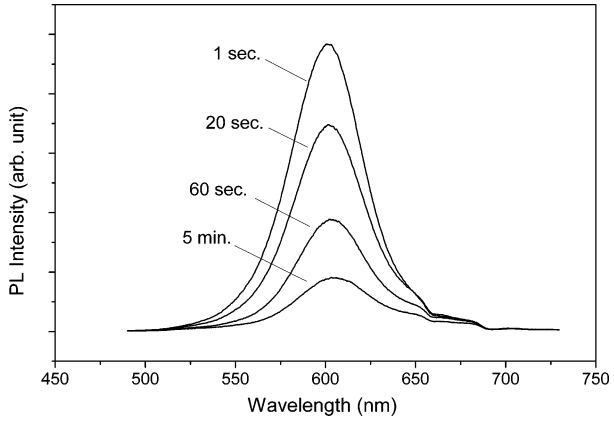

Figure 7. Time evolution of the PL spectra recorded from the QD aqueous solution with histidine added.

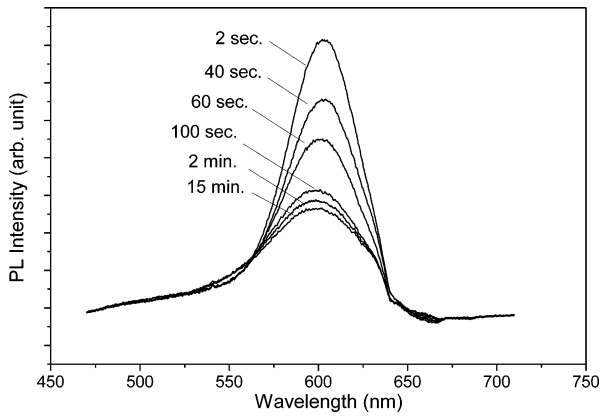

Figure 8. Time evolution of the PL spectra recorded from the QDs in a C2C12 cell treated with histidine.

Histidine is known as a scavenger of singlet oxygen ($^1O_2$). It has been revealed that histidine can react directly with $^1O_2$ at a very high reaction rate.[32−34] The effect of histidine was first studied in QD aqueous solutions. A 10 mg amount of histidine powder was dissolved in 5 mL of water to make the histidine stock solution. A 2 mL amount of QD aqueous solution (1 mg/mL) was added with the histidine stock solution to reach a histidine concentration of 3.2 mM. The irradiation power density was 3.7 kW/cm^2. The PL spectra from the histidine-added solution are illustrated in Figure 7 as a time evolution. It is distinct that no blue shift was observed.

To prepare histidine-treated cell samples, histidine stock solution was added to the cell dish containing QD-loaded C2C12 cells to reach a histidine incubation concentration of 3.2 mM. The cells were then incubated for 10 min. Figure 8 shows the time evolution of the PL spectra from the histidine-treated cells. Only a blue shift of 4 nm was observed for an illumination time of 100 s, and no further shift occurred afterward. This small blue shift was likely due to insufficient histidine−$^1O_2$ reactions inside the cell. Comparing with the blue shift of 35 nm in the C2C12 cells without histidine treatment (Figure 3A), these data (in cells as well as in solutions) reveal that singlet oxygen played a dominant role in the photooxidation of QDs. It was reported that semiconductor QDs alone could actually generate photo-

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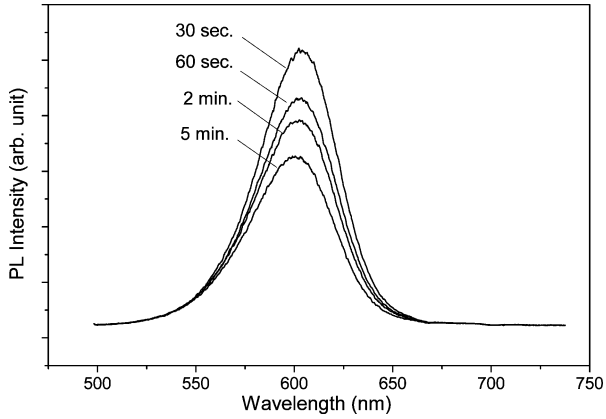

Figure 9. Time evolution of the PL spectra of the QD aqueous solution bubbled with nitrogen gas and added with histidine.

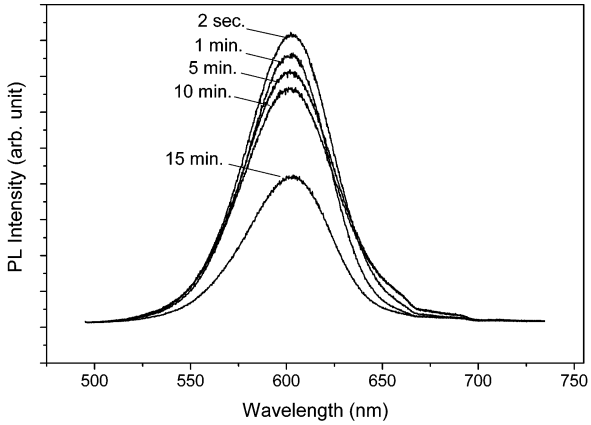

Figure 10. Time evolution of the PL spectra from the QDs in aqueous solution with NaN_3 added.

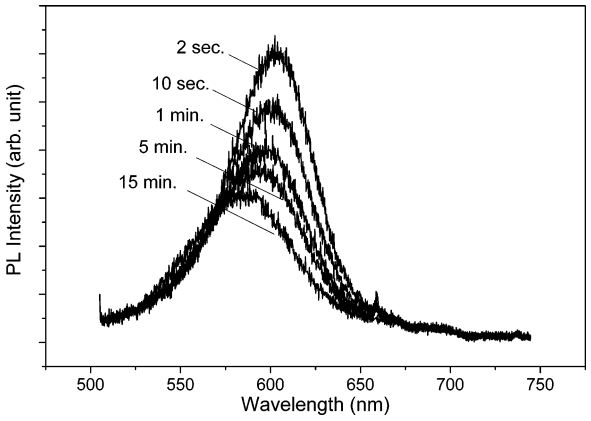

Figure 11. Time evolution of the PL spectra from the QDs in a NaN_3-treated C2C12 cell.

induced $^1O_2$ species without a mediating molecule.[35] The QDs are strong absorbers due to their large transition dipole moment. As primary energy donors, they transfer their absorbed photoenergies to the nearby oxygen molecules to form reactive singlet oxygen species, which subsequently react with the QDs to form oxides.[35] When histidine is added, it reacts rapidly with singlet oxygen and effectively hinders oxidation of QDs. Although the singlet oxygen quantum yield is low (only 5% for CdSe QDs as reported by Burda et al.[35]), in our experiments the laser beam was focused by a 60× objective and the excitation power densities used were about 250 W/cm^2 in cells and 3.7 kW/cm^2 in solutions; hence, generation of enough singlet oxygen for the localized photooxidation on the QD surface was reasonable.

However, in each of the histidine-treated samples, photobleach was still observed (Figures 7 and 8). To find out the origin of this un-blue-shifted photobleach, a QD aqueous solution was added with histidine and bubbled with nitrogen gas. In the measured PL spectra, no blue shift was observed but photobleach still occurred as shown in Figure 9. Note that this situation is entirely different from that shown in Figure 5, where the solution was also deoxygenated but histidine was not added and no photobleach was observed. It is likely that this photobleach resulted not from the photooxidation but from the histidine added. Histidine is a weak acid. The pH of the QD solution was measured to vary from 7 to 6 when histidine was added. As reported, the pH in the QD solution influenced the luminescence efficiency.[21,36] The acid in the solution caused the dissociation of organic ligands from the QD surface and therefore reduced the PL efficiency.[36] Laser irradiation might accelerate this process.

We must note that other potential reactive oxygen species (ROS) formed by a Type I photooxidation mechanism could also be responsible for the blue shift. These ROS would also react with histidine. To clarify this issue, experiments with NaN_3 were conducted in solutions as well as in cells. NaN_3 is an efficient physical quencher of $^1O_2$.[37,38] The presence of a physical quencher of singlet oxygen would not remove Type I intermediates. Figure 10 shows the time evolution of the PL spectra of a QD aqueous solution with a NaN_3 concentration of 20 mM. The irradiation power density applied was also 3.7 kW/cm^2. It is distinct that no blue shift was observed. This result definitely supports our above conclusion that the blue shift resulted from the photooxidation with singlet oxygen. Nevertheless, the PL was quenched with time. Similar quenching was also observed when the NaN_3-added QD solution was bubbled with nitrogen gas, suggesting that this kind of quenching was not associated with oxidation but most probably due to the photoinduced reaction of the organic ligands with NaN_3, although the details are still unknown.

The NaN_3 effect was also studied in cells. The C2C12 cells were incubated with QDs and NaN_3 for 4 h. The QD incubation concentration was 100 μg/mL. The incubation concentration of NaN_3 was 10 mM, which is commonly used for cells.[39,40] The time evolution of the PL spectra in a NaN_3-treated C2C12 cell is illustrated in Figure 11. The blue shift was still observed but slowed as compared with the untreated sample (Figure 3A). The blue shift was 18 nm for an irradiation time of 15 min, while it was 35 nm when the cells were not treated with NaN_3 (Figure 3A). Since there was no blue shift observed in solutions, where the NaN_3 concentration was higher, these blue shifts were most likely due to the low intracellular concentration of NaN_3, although it is difficult to measure the actual NaN_3 concentrations

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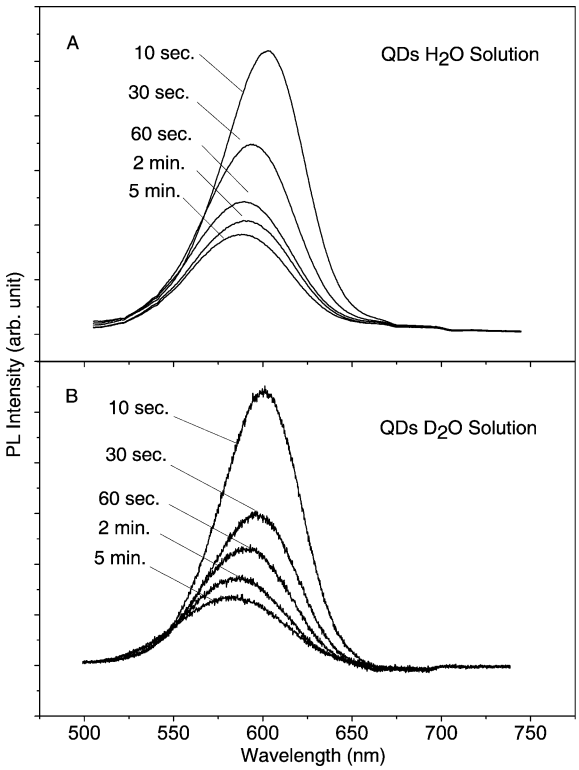

Figure 12. Time evolution of the PL spectra from the QDs in water (A) and deuterated water (B) solutions.

inside the cells. Increasing the intracellular concentration of NaN_3 may inhibit the blue shift further, but that would cause the cytotoxic effect on cells.

On the other hand, it is known that the lifetime of singlet oxygen in deuterated water (D_2O) is 10 times longer than that in water.[41] If singlet oxygen molecules are generated directly on the QD core surface, the lifetime of singlet oxygen will not be an important factor for the oxidation efficiency; otherwise, the longer lifetime would increase the oxidation probability effectively. Figure 12 shows the time evolutions of the PL spectra from QDs in water solution (A) and in deuterated water solution (B). The solutions were prepared by adding 0.1 mg of CdTe QD powders into 5 mL of H_2O and 5 mL of D_2O, respectively. It is found that the rate of blue shift in D_2O is only a little faster than that in H_2O. These data support our above hypothesis that the singlet oxygen is formed on the QD surface by energy transfer from the QDs. The O_2 molecules involved in oxidation are likely to intercalate in the thiol layer at the core surface as suggested by Burda et al.[35]

Conclusion

We have presented strong evidence that singlet oxygen is responsible for the PL blue shift of the thiol-capped CdTe QDs in living cells. The PL behavior is directly related to the existent oxygen molecules in the living cells. Deoxygenation can effectively prevent the PL blue shift from occurring. The singlet oxygen molecules are presumably formed from the oxygen molecules intercalated in the thiol layer at the QD core surface. When QDs are used as the fluorescence probes for cellular imaging, the effects of PL blue shift and photobleach must be considered.

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