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A Nonenzymatic Chemiluminescent Reaction Enabling Chemiluminescence Resonance Energy Transfer to Quantum Dots

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Resonance energy transfer (RET)-based measurement approaches are very useful for innovative studies, such as that of protein–protein interactions in living cells with spatial and/or temporal resolution.[1] RET involves nonradiative (dipole–dipole) energy transfer between a donor and an acceptor that are in close proximity (normally <10 nm). RET that occurs between two fluorophores is known as fluorescence RET (FRET), whereas RET that occurs between a light-emitting donor enzyme (e.g., luciferase) and a fluorophore is known as bioluminescence RET (BRET). Numerous publications exist for FRET being used in various areas, such as structural elucidation of biological macromolecules, their interactions, in vitro assays, in vivo monitoring, and signal transduction in living cells.[2,3] BRET is also well documented as a technique useful for these studies.[4,5] Major disadvantages of BRET include the requirement of at least one carefully designed protein fusion and the low emission intensity that compromises the spatial and/or temporal resolutions in BRET measurements. Chemiluminescence RET (CRET) involves nonradiative transfer of energy from a chemiluminescent (CL) donor (instead of a bioluminescent enzyme donor as in BRET) to a fluorophore acceptor.[6] CRET occurs by the oxidation of a CL compound that then excites the fluorescent acceptor. Since no external light source is used for excitation in CRET approaches, nonspecific signals caused by external light excitation as often observed in FRET measurements can be minimized. Compared with BRET, a CRET-based approach involves no protein fusion. Both the CL donor and fluorescent acceptor can be conjugated to antibodies, which promises widespread application. However, little study has been reported, so far, on CRET.[6–8] A major difficulty is to identify an effective CL donor or reaction that can excite a fluorescent acceptor by energy transfer. In all of the previous CRET works reported, the luminol-H_2O_2 CL reaction catalyzed by horseradish peroxidase (HRP) was used. Unfortunately, involving an exogenous enzyme (i.e., HRP) limits the applicability of the CRET system. In many cases, it complicates the assay by, for example, disturbing the biological interactions under study.

Luminescent semiconductor nanocrystals (also called quantum dots, QDs) have optical properties of a broad excitation spectrum: sharp and symmetrical emission spectra, high quantum yield, good chemical and photostability, and size-dependent emission-wavelength tunability. They are suitable materials for developing RET approaches. QDs have been used as both donors[9,10] and acceptors[11,12] in FRET. In recent years, FRET protocols involving QDs have been developed to probe DNA replication and telomerization,[13] to study enzymatic activity and enzyme inhibitors,[14] to make DNA nanosensors,[15] and to facilitate photodynamic medical therapy.[16] QDs have also been used as energy acceptors in BRET measurements.[17]

Herein, we report on a novel nonenzymatic luminol CL reaction that enables CRET with luminescent CdTe QDs as the energy acceptor. We comparatively studied various luminol CL reactions utilizing different oxidizers, such as H_2O_2 or $KMnO_4$. It was found that when NaBrO was used as the oxidizer, CRET occurred between a luminol donor and a CdTe QD acceptor. No HRP catalysis on the luminol CL reaction was needed. Figure 1 illustrates the CRET principle. Effects of reactant concentrations on the CRET intensity were studied. It was noted that the CRET intensity increased with the increase in the concentration of luminal (or QDs) and the reaction could be recharged by adding more luminol into the system.

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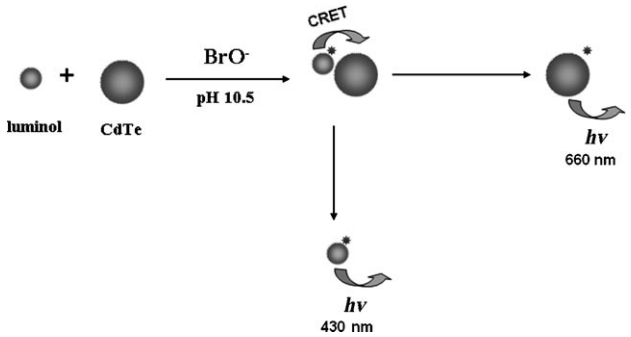

Figure 1. Illustration of CRET between a luminol donor and a CdTe QD acceptor.

Luminol–H_2O_2 CL reaction is one of the most sensitive CL reactions and widely used in CL bioassays. Luminol CL reaction can be catalyzed by HRP and the catalytic CL emission can be further enhanced by a CL enhancer, such as *para*-iodophenol (*p*-IP). As shown in Figure 2, there is an

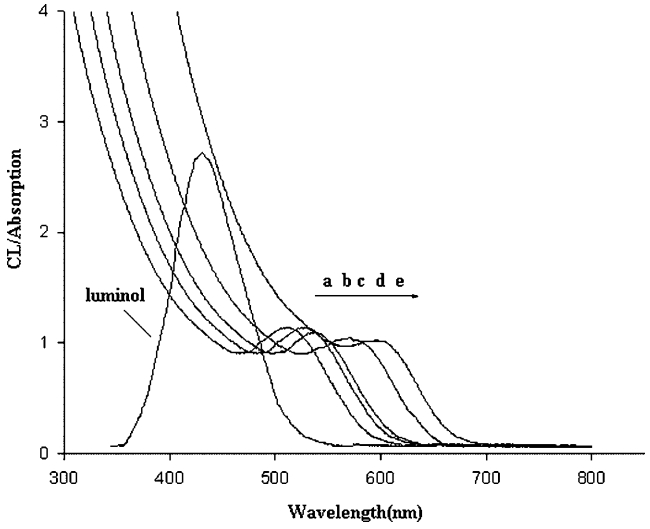

Figure 2. Luminol CL spectrum and the absorption spectra of CdTe QDs of different sizes: a) 2.31 ($\lambda_{max}=550$), b) 2.93 (572), c) 3.82 (596), d) 4.08 (614), and e) 4.41 nm (660 nm). The CL reaction buffer (pH 10.5) was 30 mM borate solution containing 1.5×10^{-4} M luminol and 5.0×10^{-5} M CdTe QDs. The concentration of NaBrO in borate buffer (pH 10.5) was 1.5×10^{-4} M.

overlap between the luminol CL spectrum (with a maximum at 430 nm) and the absorption spectra of CdTe QDs of various sizes. This satisfies the essential condition for CRET. However, in our studies no CRET between luminol–H_2O_2 and CdTe QDs was observed (as shown in Figure 3). This finding confirms the results reported previously by Huang et al..[6] They described that CRET was not observed from a solution containing luminol, H_2O_2, and CdTe QDs. However, an efficient CRET was observed from a solution containing luminol, H_2O_2, *p*-IP, and HRP-conjugated CdTe QDs. Apparently, conjugating the CL catalyst (HRP) to the

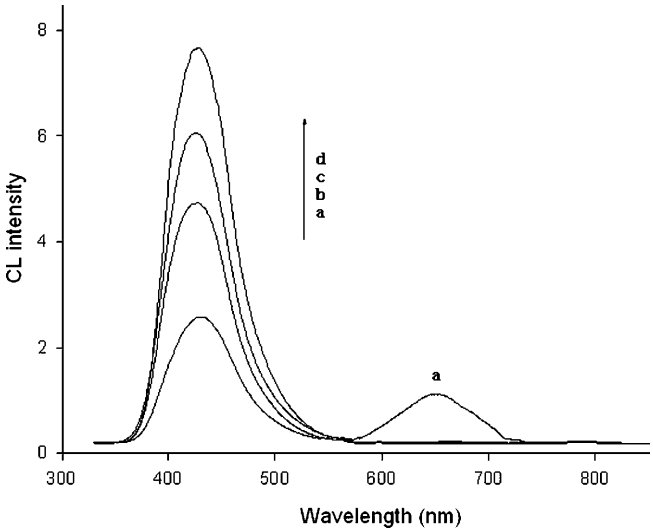

Figure 3. CL spectra obtained from luminol–oxidizer–QD (4.41 nm) systems with different oxidizers: a) NaBrO, b) H_2O_2, c) $K_3Fe(CN)_6$, and d) $KMnO_4$. Experimental conditions were the same as those given in Figure 2.

energy acceptor (DQs) enhances its interaction with the energy donor (oxidized luminol molecules). It is worth noting, however, the requirement of labeling the energy acceptor with a specific protein limits the application of this CRET system. To solve this problem, we investigated various oxidizers, including H_2O_2, $K_3Fe(CN)_6$, $KMnO_4$, and NaBrO. These compounds are effective to oxidize luminol, producing CL emission. Figure 3 shows the results obtained from four different luminol–oxidizer–QD systems. Interestingly, an efficient CRET was observed from the luminol–NaBrO–QD system for the first time. The CRET ratio calculated by dividing the acceptor emission by the donor emission was comparable to that observed from the luminol–H_2O_2–HRP–conjugated QD system, that is, ≈30%. No other oxidizers tested induced CRET between luminol and CdTe QDs. The mechanism of inducing CRET by NaBrO is unknown. There is a possibility that an intermediate complex is formed between BrO^-anions and CdTe QDs, which bring the energy donor (luminol molecules) and the acceptor (QDs) very close to each other at the time of oxidation to produce chemiluminescence emission. However, no conclusion can be drawn before further experimental results confirm this reasoning. It should be noted that in the luminol–NaBrO–QD system native CdTe QDs are used as the energy acceptor. Therefore, CRET approaches based on this system with antibody-conjugated CdTe QDs promise to be as useful as FRET approaches in chemical and biological studies, and in the meantime offer CRET advantages, such as a superior signal-to-noise ratio.

We investigated the effects of QD diameter on the CRET. QDs with diameters of 2.31, 2.93, 3.82, 4.08, and 4.41 nm were tested. The photoluminescent emission maxima were recorded at 550, 572, 596, 614, and 660 nm, respectively, for these differently sized QDs. Figure 4 shows the CL spectra

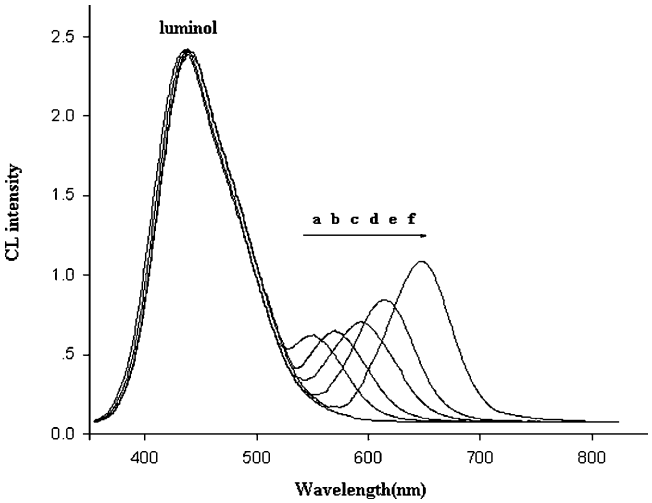

Figure 4. CL spectra of luminol–NaBrO solutions containing QDs of different sizes: a) no QDs, b) 2.31 nm ($\lambda_{max}=550$), c) 2.93 nm (572), d) 3.82 nm (596), e) 4.08 nm (614), and f) 4.41 nm (660 nm). Experimental conditions were the same as those given in Figure 2.

obtained from luminol–NaBrO–QD mixture solutions with differently sized QDs. As can be seen, the acceptor emission spectra match the photoluminescent emission spectra of QDs. The large Stoke's shift shown in this CRET system (the largest one is 230 nm for 4.41 nm QDs) warrants distinct separation of excitation and emission wavelengths and, thus, a superior signal-to-noise ratio in the CRET measurements. The CL emission from a solution containing two different sized QDs (2.93 and 4.41 nm) was studied. The CL spectra are shown in Figure 5. As can be seen, two acceptor emission peaks at 572 and 660 nm appear in the spectra. These results indicate that the luminol donor can transfer

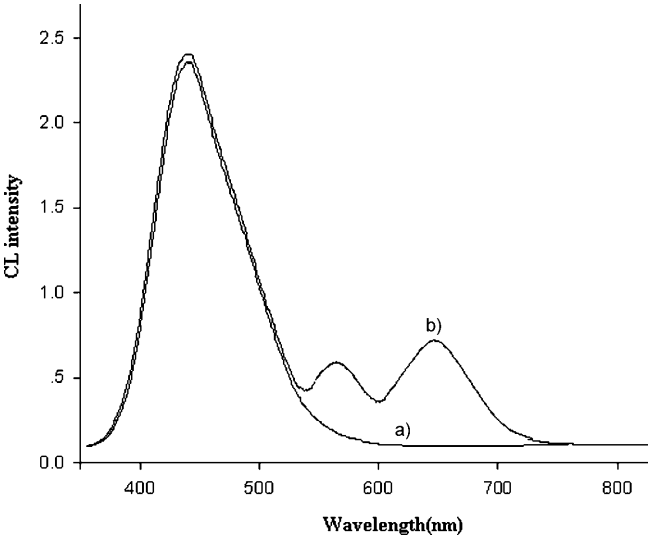

Figure 5. CL spectra of a luminol–NaBrO solution containing two differently sized QDs: a) a luminol–NaBrO solution; b) a solution containing luminol, NaBrO, 2.93 nm QD ($\lambda_{max}=572$ nm), and 4.41 nm QD ($\lambda_{max}=660$ nm). Experimental conditions were the same as those given in Figure 2.

energy to multiple QD acceptors at the same time, that is, multiplexed CRET occurs in this system. This will allow applications similar to those of multiplexed FRET.[18]

We studied the effects of reactant concentrations on the CRET. Solutions containing QDs at 5.0×10^{-5} M and luminol at concentrations varying from 5.0×10^{-5} to 2.5×10^{-4} M were prepared. CL spectra were recorded from each of the solutions immediately after adding NaBrO. Figure 6a–e show

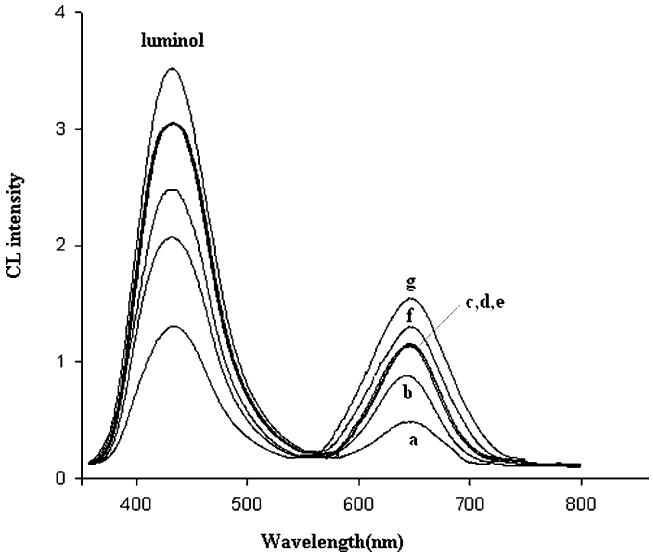

Figure 6. CL spectra of the luminol–NaBrO–QD solutions containing QDs at 5.0×10^{-5} M, NaBrO at 1.5×10^{-4} M, and luminol at different concentrations: a) 5×10^{-5}, b) 1.0×10^{-4}, c) 1.5×10^{-4}, d) 2.0×10^{-4}, and e) 2.5×10^{-4} M. f) A solution containing 2.0×10^{-4} M luminol and 7.0×10^{-5} M QDs, and g) is a solution containing 2.0×10^{-4} M luminol and 9.5×10^{-5} M QDs. All spectra were obtained by using 4.41 nm QDs. Other experimental conditions were the same as those given in Figure 2.

the results. As can be seen from Figure 6a–c, both luminol CL emission and the CRET intensity increased with the increase in luminol concentration until it reached 1.5×10^{-4} M. At this point, QDs became the "limiting reactant", that is, the quantity of the energy acceptor determined the CRET intensity although luminol CL emission continued to increase as its concentration increased (Figure 6c–e). If the acceptor quantity was increased, a further increase in CRET intensity was observed (Figure 6 f and g). The CRET ratios were calculated as 23.5, 30.4, 32.6, 27.2, 24.6, 31.3, and 34.4% for Figure 6a–g, respectively.

Changes of luminol CL and the CRET emission depending on the scanning time were also investigated. Consecutive scans were performed on a solution containing 1.5×10^{-4} M luminol, 5.0×10^{-5} M CdTe QDs, and 1.5×10^{-4} M NaBrO. The results are shown in Figure 7. Luminol CL emission showed a $\approx$70% decrease and the CRET intensity a $\approx$90% decrease 100 s after starting the CL reaction. Interestingly, luminol CL and the CRET emissions recurred when fresh luminol and NaBrO were added into the solution. This indicated that the reaction was rechargeable.

In summary, we report herein the first observation of an efficient CRET between a luminol donor and a CdTe QD

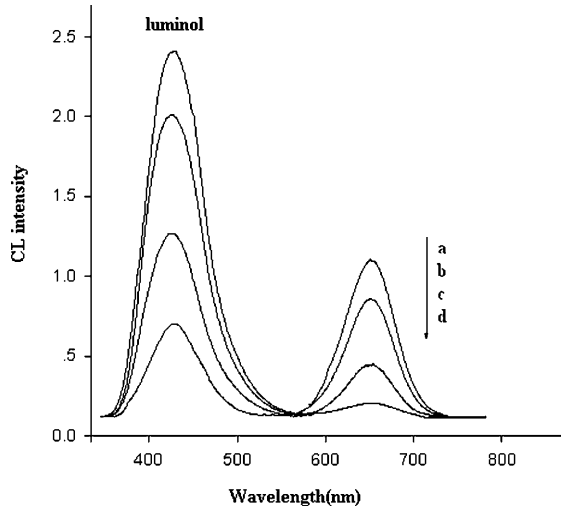

Figure 7. CL spectra obtained from a solution of luminol–NaBrO–QDs by repetitively scanning. Each scan took about a) 20, b) 50, c) 75 and d) 100 s. The solution contained 1.5×10^{-4} M luminol, 5.0×10^{-5} M QDs (4.41 nm), and 1.5×10^{-4} M NaBrO. Other experimental conditions were the same as those given in Figure 2.

acceptor in the absence of an enzyme catalyst. CRET occurs when the right oxidizer is used in the luminol CL reaction. Among the oxidizers tested, that is, H_2O_2, $K_3Fe(CN)_6$, $KMnO_4$, and NaBrO, only the luminol–NaBrO CL reaction enables CRET with luminescent QDs as the energy acceptor. Both luminol CL emission and the CRET intensity were found to be dependent on the concentrations of reactants, that is, luminol, QDs, and NaBrO. After the CL reaction started, the CRET emission decreased quickly (up to 90% within ≈100 s). However, the reaction was rechargeable by adding fresh CL reactants into the system. The CRET ratio calculated by dividing the acceptor emission by the donor emission was approximately 30%. Since no enzyme is involved, the new luminol–NaBrO–QD CRET system will promote the widespread application of CRET in chemical and biological research. It is our expectation that this CRET system will become as useful as FRET systems for innovative studies such as that of protein–protein interactions in living cells and offers CRET advantages such as a superior signal-to-noise ratio.

Experimental Section

CdTe QDs were prepared by using a procedure similar to the method described by Li et al.[19] with minor modifications. Ar-saturated cadmium chloride solution was added to NaHTe solution, which was prepared by the reaction between $NaBH_4$ and tellurium powder in the presence of mercaptopropyl acid (MPA). The concentration of Cd^{2+} was 2 mM, and the molar ratio of Cd^{2+}/Te^{2-}/MPA was fixed at 1:0.5:2.5. After being mixed the solution was heated with a microwave at different reaction times to prepare different-sized QDs. The prepared CdTe QDs were purified by selective precipitation with isopropanol and redispersed in ultrapurification water. The CdTe QDs were further purified by dialysis (dialysis membrane with a molecular weight cutoff at 7000) in 10 mM NaOH. The concentration of MPA-capped CdTe QDs was defined by the number of cadmium atoms contained in the sample. The particle size distribution was obtained by a NaNo-ZS90 particle size and Zeta potentiometer analyzer.

CL emission spectra were recorded with a LS-55 luminescence spectrometer (Perkin–Elmer, USA) using a 3 mL quartz call (1 cm optical path). To carry out the luminol-NaBrO CL reaction, the reaction buffer (pH10.5) consisting of 30 mM borate buffer solution, 1.5×10^{-4} M luminol, and 5.0×10^{-5} M CdTe QDs was transferred to the quartz cell. NaBrO (prepared in pH 10.5 borate buffer) was then added to the cell at a concentration of 1.5×10^{-4} M. CL emission spectra were recorded immediately after the addition.

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Keywords: CRET (chemiluminescence resonance energy transfer) · energy transfer · enzymes · luminol · quantum dots

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