Research Article

A Turn-On Fluorescent Probe for Detecting Nitric Oxide in Aqueous Media

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Imaging of nitric oxide (NO) in living cells can provide new insights in understanding unsolved pathways in the cardiovascular and neurological dysfunction and many types of cancers. For the detection of NO under physiological conditions, we synthesized a turn-on fluorescent probe comprising CdSe/ZnS quantum dots (QDs) coordinated with dirhodium complex. Emission from CdSe/ZnS-QDs is quenched when coordinated with the dirhodium complex but can be restored upon the displacement by nitric oxide.

Since nitric oxide (NO) was described as an endothelium-derived relaxing factor in 1987 [1, 2], it has been well established that NO is a ubiquitous messenger molecule in the cardiovascular, nervous, and immune systems and tumor progression [3–5]. In addition, NO is known as a double edged sword in many diseases, especially cancer, because of its roles of an uncharged free radical as well as a radical scavenger [6]. The exact function is depending on its temporal and spatial distribution in the body. However, NO is highly reactive and it can be converted to other species quickly; thus it is very difficult to detect and monitor the concentration of NO in vivo. Fluorescence-based approach has been shown in meeting the requirement to monitor NO molecule directly in the biological systems [7–11]. Also, Rhodamine-based fluorescent probes for detecting ions have been of interest in living cell imaging [12–14]. Herein, we describe new QDs-based nanohybrids incorporated with transition metal complexes that sense NO by turn-on emission with nanomolar sensitivity in aqueous media.

Nagano and Yoshimura developed NO probes containing organic fluorophores, in which the fluorescence of the organic probe was turn-on upon oxidation by dinitrogen trioxide (N_2O_3) formed from the NO oxidation in the presence of oxygen [15]. Lippard and coworkers reported that a fluorescein-based Cu (II) complex probe can detect NO with the

sensitivity of nanomolar concentration [16, 17]. However, they do not react directly with NO molecule but monitor products of its oxidation. Furthermore, these probes cannot detect and monitor their temporal concentration but accumulated concentration in the biological systems because of their irreversibility. Wang et al. reported that inorganic QDs were coordinated iron complex for detecting NO selectively by turn-on emission with the limit of detection (LOD) of $\sim 3 \mu\text{M}$ which is not enough in the biological systems [18].

A fluorescent chemo- and biosensor for NO detecting such as QD displacement without metal reduction were not reported yet. The development of fluorescent probes that are stable and significantly brighter than traditional organic fluorophores has been of considerable interest to realize biosensors with high sensitivity [19, 20]. Semiconductor QDs are usually described as fluorophores having remarkable photostability, large absorption cross section, and tunable emission peaks [21, 22]. Furthermore, they have the better optical properties with tunable photoluminescent emissions and exceptional resistance to both photobleaching and chemical degradation.

In this work, we first report a turn-on fluorescent probe with nanomolar sensitivity to NO comprising CdSe/ZnS QDs coordinated with dirhodium complex. Emission from CdSe/ZnS-QDs is quenched when coordinated with the

SCHEME 1

FIGURE 1: (a) FE-TEM micrographs of and (b) UV-Vis absorption and fluorescence emission spectra of CdSe/ZnS-QDs.

dirhodium complex but can be restored upon the displacement by nitric oxide as shown in Scheme 1. This suggested mechanism should be supported by the further mechanistic and theoretical studies. Figure 1 shows (a) FE-TEM micrographs of CdSe/ZnS-QDs and (b) UV-Vis absorption and fluorescence emission spectra in this work. The CdSe/ZnS-QDs were successfully obtained with about 3 nm diameter of spherical nanoparticles as observed in Figure 1(a). The green fluorescence emission of CdSe/ZnS-QDs was observed ca. 530 nm of wavelength as shown in Figure 1(b). Based on the quantum yield of Rhodamine 6G as a reference, the report shows that the quantum yield of CdSe reached 58% [23]. Figure 2 shows quenching behavior of fluorescence spectra of the 30 nM CTAB-capped CdSe/ZnS-QDs when mixed with 300 nM $\text{Rh}_2(\text{AcO})_4$ at 40°C in aqueous media. The CdSe/ZnS QDs were quenched with increasing reaction time. Initially, the fluorescence of CdSe/ZnS-QDs was rapidly decreased for 30 min but was no longer decreased after 7 hours. Figure 3 shows the intensity change of photoluminescence spectra of the 30 nM CdSe/ZnS-QDs containing 300 nM dirhodium complex according to the addition amount of

DEA/NO (diethylamine NONOate, 2-(N,N-diethylamino)-diazonolate-2-oxide, and diethylammonium salt). DEA/NO is a complex of diethylamine with nitric oxide used to generate a controlled release of nitric oxide in solution.

Upon adding 3000 nM DEA/NO, the fluorescence intensity of the CdSe/ZnS-QDs containing dirhodium complex was switched on and reached its maximum immediately. As shown in Figure 3, the CdSe/ZnS-QDs containing dirhodium complex in aqueous media showed ca. 80.3% increase in relative fluorescence emission when adding to 100 equiv. of DEA/NO. In the case of 50 and 10 equiv. of DEA/NO, the relative intensity of fluorescence emission showed ca. 48.5% and 27.3% increase, respectively.

This result suggests that the probe can detect NO with nanomolar sensitivity much better than previous works. NO sensors comprising transition metal complexes coordinated with organic fluorophores were reported utilizing cobalt [24], ruthenium [25], and dirhodium complex [26]. But these systems are limited by low sensitivity ($\sim 4 \mu\text{M}$) and their lack of water compatibility in the biological systems. However, we need to carry out the selectivity experiment

FIGURE 2: The intensity change of photoluminescence spectra of solutions mixed with the CTAB-capped CdSe/ZnS-QDs and dirhodium acetate as a function of the reaction time.

FIGURE 3: The intensity change of photoluminescence spectra of the CdSe/ZnS-QDs containing dirhodium complex as a function of the concentration of DEA/NO.

for this fluorescent probe including the dirhodium complex even though the probe consisting of densyl piperazine dye coordinated with dirhodium complex shows the excellent selectivity to NO [27]. Further studies will be performed to figure out whether the probe is interfered with other species such as HOCL, H_2O_2 , tBuOOH, and cysteine. In order to investigate the reversibility of the probe, the process adding DEA/NO solution to CdSe/ZnS-QDs coordinated dirhodium complex was carried out twice. The ratio of QDs : dirhodium complex : DEA/NO remains the same as 1 : 10 : 50. In the first step, 2 mL of 1500 nM DEA/NO solution was injected into

4 mL of 45 nM CdSe/ZnS-QDs containing dirhodium complex. Then N_2 in the solution was purged for 30 min to take NO molecule off the surface of CdSe/ZnS-QDs. After that, 2 mL of 1500 nM DEA/NO solution was injected into 4 mL of 30 nM CdSe/ZnS-QDs containing dirhodium complex again. As shown in Figure 4, the relative fluorescence intensity of “turn-on” state after the first and second step showed 54.2% and 27% increase, respectively. And the relative intensity of “turn-off” state before injecting DEA/NO solution is 6.4% and 2.9%, respectively. It should be noted that the ratio of “turn-on” to “turn-off” shows 8.46 and 9.3, respectively. This

FIGURE 4: Photoluminescence spectra of the CdSe/ZnS-QDs containing dirhodium complex with repetitious addition of DEA/NO solution.

result suggests that the CdSe/ZnS-QDs nanohybrid can be used for the recyclable turn-on sensors for NO detection. This finding is very important because the probe must be reversible for monitoring the real concentration specially and temporally in the biological systems.

In summary, we first demonstrated here a “turn-on” reversible fluorescence probe with nanomolar sensitivity. Addition of DEA/NO solution to the aqueous solution of QDs nanohybrids affords a substantial increase in fluorescence intensity reversibly. The result shows that the nanohybrids turn on with nanomolar sensitivity upon the displacement of dirhodium complex by nitric oxide. These results suggest that the nanohybrids can be used for monitoring and imaging NO concentration directly in biological systems with a “turn-on” manner.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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