J|A|C|S COMMUNICATIONS

Published on Web 03/17/2007

Photosensitized NO Release from Water-Soluble Nanoparticle Assemblies

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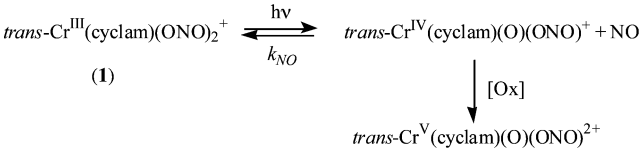
There is active biomedical interest in developing methodologies for delivering the bioregulatory diatomic nitric oxide[1] (NO) to physiological targets for applications ranging from cardiovascular control to radiation sensitization.[2] Photochemical techniques provide the opportunity to control the location and timing of the signal leading to NO release. In this context, we and others have been studying the photoreactions of transition metal nitrosyl and nitrito complexes with the goal of developing thermally stable precursors that release NO upon electronic excitation.[3] One direction that we have taken is the preparation of molecular constructs consisting of the NO donor plus an antenna chromophore to collect the light via single photon excitation (SPE)[4] or two photon excitation (TPE).[5] Energy or electron transfer from the antenna to the NO precursor would then lead to net NO release.

Nanocrystal quantum dots offer certain important advantages as photosensitizing chromophores, including high optical cross-sections for both SPE[6] and TPE[7] and the ability to tune the optical properties by varying the QD diameter.[8] Multiple surface ligand sites provide the opportunity to incorporate functionalities tethered to the QD surface, such as solubility properties,[9] biological specificity,[10] and the NO precursor or carrier.[11] Furthermore, functionalized QDs have been used as sensitizers for organic reactions[12] for singlet oxygen generation[13] and for electron transfer.[14] Here we demonstrate enhanced NO photogeneration from electrostatic assemblies between water-soluble CdSe/ZnS core/shell QDs and the cationic complex *trans*-Cr(cyclam)$(ONO)_2^+$ (**1**, cyclam = 1,4,8,11-tetraazacyclotetradecane), indicating that the QDs are serving as antennas to sensitize photoreactions of **1**.

The dinitrito complex **1** (BF_4^- salt) is photoactive toward cleavage of the CrO−NO bond to give NO plus a $Cr^{IV}O$ intermediate (Scheme 1).[4b,15] However, **1** is relatively weakly absorbing at near-UV and visible wavelengths, thus rates of NO production from this photochemical precursor would be enhanced by an antenna that increases the rate of light absorption. From this derives our interest in QDs as sensitizers.

Scheme 1



The water-soluble quantum dots were prepared by a three-step procedure involving synthesis of the CdSe cores,[16] growth of the ZnS shell,[17] and surface ligand exchange as described in the Supporting Information. The average CdSe core diameter (3.8 nm) was estimated from the 542 nm peak position (fwhm = 33 nm) of the first excitonic transition.[18] A ZnS shell of ∼6 monolayers was grown, and surface ligands were exchanged with dihydrolipoic acid (DHLA)[19] to give aqueous solubility in moderately basic solution. After purification, the result was a QD stock solution in aqueous phosphate buffer (PB) solution (15 mM, pH 8.2) that displayed a 570 nm photoluminescence (PL) maximum (fwhm = 48 nm) with Φ_{PL} = ∼2% (Figure 1).

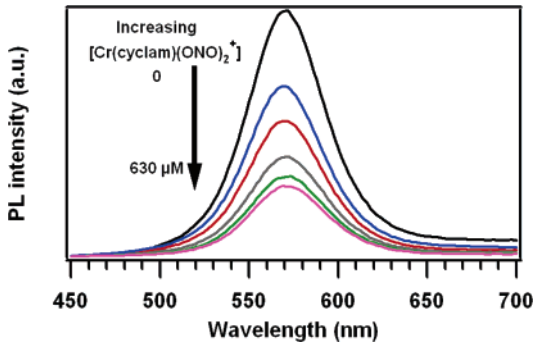
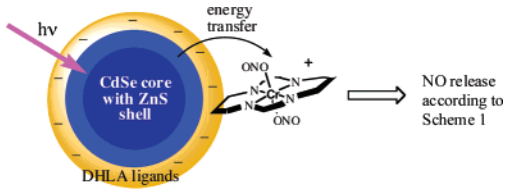


Figure 1. PL spectra (λ_{ex} 366 nm) of water-soluble QDs (∼130 nM) in phosphate buffer (15 mM, pH 8.2) with various concentrations of added **1** (0−630 μM). PL intensities were corrected for the absorbance by **1**. The PL λ_{max} shifts slightly (∼3 nm) to the red at the highest value of [**1**].

Scheme 2



When various concentrations of **1** were added to the QD solution (∼130 nM),[20] the PL intensity from the QDs decreased (Figure 1). This quenching levels out at ∼60% for the highest value of [**1**] (630 μM). Analysis of similar QD PL partial quenching by the analogous *trans*-Cr(cyclam)Cl_2^+ complex led us to conclude[21] that the quenching is due to electrostatic ion pairing between the cationic Cr(III) complex and the anionic QD surface, as illustrated in Scheme 2. Although either energy or electron transfer from the excited QD to Cr(III) cations at the surface would account for the quenching seen with varied [**1**], the photostimulated release of NO (below) parallels the excited-state chemistry of **1** (Scheme 1)

The QD PL quenching is also accompanied by enhanced photoreaction of the Cr(III) complexes. This was determined by using a NO-specific electrode immersed in a cuvette filled with stirred PB solution irradiated by the continuous output of a high-pressure Hg arc lamp (spectrally selected by a 320−390 nm band-pass filter). When a small volume of **1** (also dissolved in PB solution) was injected into the cuvette solution to give an initial concentration of 200 μM, the electrode response due to photo-

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10.1021/ja070490w CCC: $37.00

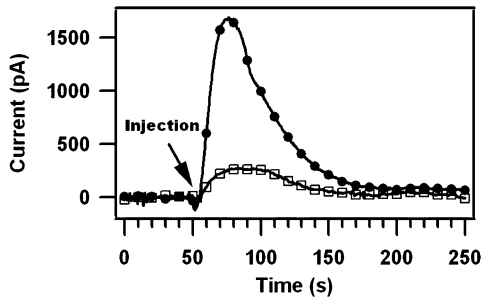

Figure 2. Detection of NO photochemically produced from **1** (200 μM) in stirred buffer solutions (15 mM, pH 8.2) with (circles) and without (squares) added QDs (100 nM). At the time designated, a 100 μL aliquot from a stock solution of **1** was injected into the stirred solution. The excitation source was a Hg arc lamp (320−390 nm band-pass filter), and NO was detected with an amiNO−700 Innovative Instruments electrode.

chemical NO generation was small (Figure 2). However, the otherwise identical experiment where the cuvette PB solution also contained water-soluble QDs (100 nM) gave a dramatically stronger NO response. While, in both cases, introduction of **1** to the solution being irradiated results in a positive response, the maximum of the NO-dependent signal was considerably higher (~6-fold) when the QDs were present. Notably, addition of QDs alone to the irradiated buffer solution (data not shown) did not elicit a response from the electrode. These results indicate that NO production from **1** is much faster when the QD antennas are present due to the enhanced light harvesting ability of that system (Scheme 2).

The effect of the QDs in enhancing photochemical NO production can be attributed to the dramatically higher extinction coefficients of the QDs ($>10^3$ as large as for **1**) as illustrated in the Supporting Information. For the experiment described above, the absorbance is dominated by the QD chromophores across the excitation wavelength range, where they absorb 2−10 times as much light as does **1**, despite the huge concentration differences (100 nM vs 200 μM). The enhanced NO photoproduction from **1** indicates that optical excitation of the QDs results in photosensitization of **1**.

These results demonstrate that QDs can function effectively as antennas for photochemical reactions of complexes that are electrostatically bound near the QD surfaces. This brings us a step closer to using such photoactive transition-metal-based NO donors as prodrugs. The current study represents the first example using water-soluble semiconductor QDs as photoactivators for drug delivery that targets the hypoxic environment often encountered in tumor cells.[2b] In vivo applications will likely require long wavelength excitation (red or near-infrared) for effective tissue penetration, possibly via TPE methods for which QDs are well suited. Furthermore, recent developments utilizing QDs for bioimaging show that specific targeting strategies can be effective.[10] In these contexts, modified QDs have considerable promise as site-specific agents for photochemical drug delivery, and the present paper demonstrates that QDs can serve as the sensitizers for photochemical NO delivery. Further studies to develop QD/NO donor constructs, including those involving covalent linkages between these components for potential application as photochemical drugs, are continuing in this laboratory.

Acknowledgment. These studies were supported by a National Science Foundation grant to P.C.F. (CHE-0352650), by a UC-CARE grant from Los Alamos National Laboratory to P.C.F. and G.F.S., and a National Institutes of Health grant (NBIB 7 R01 EB000832) to G.F.S. We thank Roberto da Silva for stimulating discussions and technical help.

Supporting Information Available: Experimental procedures for preparation of QDs and for evaluating NO photoproduction. Figure comparing the solution spectra of **1** and the QDs. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA070490W