Functionalized Selenium-Doped Carbon Quantum Dots for Targeting Detection of Reactive Oxygen Species in Live Cells

Danling Zhou¹, Hong Huang², Yan Wang¹, Junrong Yu¹ and Zuming Hu¹, *

¹State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, China
²College of Biological, Chemical Sciences and Engineering, Jiaxing University, Jiaxing 314001, China

*Corresponding author: hzm@dhu.edu.cn

Abstract. Selenium doped carbon quantum dots (Se-CQDs) are synthesized by a facile hydrothermal process. The Se-CQDs show narrow size distribution in the range of 3-4 nm, good optical properties, and high sensitivity in detection of various reactive oxygen species (ROS) as revealed by several characterizations. The Se-CQDs are then modified by three kinds of organelle targeting molecules with the aim of targeting detection of ROS in specific organelles. The confocal fluorescence studies on modified Se-CQDs and commercial organelle marker demonstrate the successful targeting detection of ROS in different organelles by our specific modified Se-CQDs.

Keywords: Reactive oxygen species, selenium, carbon quantum dots, targeting detection.

1. Introduction

Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), hypobromous acid (HBrO), hypochlorous acid (HClO), superoxide radicals (O₂⁻), hydroxyl radicals (·OH), and singlet oxygen (¹O₂) [1, 2], play an important role in many physiological processes, such as the immunity, homeostasis, regulation, and signal transduction. However, excess amount of ROS would cause many diseases such as diabetes, neurological disorders, and even cancer cell development in human body. The damage to people’s health is further dependent on the location and responses of ROS in cells [3-5]. Therefore, it is highly necessary to exploit characterization methods or proper tools to monitor the development of ROS in organelles of live cells.

Currently, a variety of approaches such as chemiluminescence method, high performance liquid chromatography, spectrophotometric method, electrochemiluminescence method, and fluorescence analysis, have been developed to detect ROS. Among these techniques, fluorescence-based methods are advantageous in aspects of high sensitivity, accurate spatial and temporal resolution, non-destroy of cellular samples, and the need less of sophisticated instrumentation [6, 7]. Various fluorescent molecules or nanomaterials have been synthesized and employed to detect and clear ROS in the past years. These synthetic materials include perylene derived molecules [8], manganese oxide nanoparticles [9], cerium oxide nanoparticles [10], MOFs [11], carbon nanomaterials [12, 13], and so
on. However, these materials are more or less suffered from tedious synthetic procedure, high cost, or cytotoxicity.

Carbon quantum dots (CQDs) are a new kind of fluorescent materials that are frequently used in bio-related fields because of their good biocompatibility, low cost and environment-friendly synthetic procedure. As such, CQDs have also been applied in bio-sensing as well as antioxidant for scavenging of free radicals in live cells [14]. The facile fabrication process of CQDs also allows the doping of hetero atoms that endows CQDs with versatile functionalities. For instance, the doping of selenium (Se) in CQDs is reported to confer redox-dependent reversible fluorescence to CQDs, these Se doped CQDs (Se-CQDs) show good free radical scavenging ability because of the function of Se in antioxidant defense in a range of biological processes [15]. However, rare work has reported the targeting detection of ROS in live cells by Se-CQDs. Inspired by the above-mentioned situation, here we reported the synthesis of Se-CQDs by a facile hydrothermal process and the functionalization of Se-CQDs with various kinds of organelle specific targeting molecules. These functionalized Se-CQDs are then applied for targeting detection of ROS in several kinds of organelles.

2. Experimental Section

2.1. Chemicals

L-Selenomethionine was purchased from J&K Scientific Ltd. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), phorbol 12-myristate 13-acetate (PMA), and three kinds of targeting molecules 1-3 (Figure 1) were supplied by Aladdin Chemistry Co. Ltd. Other reagents were all bought from KeyGEN Biotech. Co. Ltd. All these chemicals were of analytical grade and used without further treatment.

2.2. Synthesis of Se-CQDs

A hydrothermal process was employed to prepare Se-CQDs. In brief, selenocystine (0.3 g) was dissolved in 35 ml deionized water, the pH of solution was then adjusted to about 10 by 0.1 M NaOH. The solution was then heated at 60 °C for 20 h under nitrogen atmosphere. After that, the products were collected by centrifugation and purified by dialysis.

2.3. Functionalization of Se-CQDs

Amidation reaction was used to modify the surface of Se-CQDs with molecules 1-3. In a typical modification process, a solution of 1 (1.0 mL, 3.5mM) was mixed with Se-CQDs solution (1, 5 mg/mL), then 80 mg EDC and 80 mg NHS were added into the mixture as catalysts. The mixture was reacted at room temperature for 12 h to obtain the functionalized Se-CQDs. The final product was purified by dialysis.

2.4. Characterizations

Transmission electron microscopy (TEM) images were obtained using JEM-2100 with acceleration voltage of 200 kV. Atomic force microscopy (AFM) characterizations were carried out using a digital Nanoscope IIIa atomic force microscope. X-ray photoelectron (XPS) spectra were recorded on an ESCA LAB 250 spectrometer (VG Scientific). UH5300 spectrophotometer was used to obtain UV–Vis data. Electron paramagnetic resonance (EPR) studies were conducted on a Bruker ELEXSYS
E500 EPR spectrometer. Confocal fluorescence and bright field images (512×512 pixels) were captured with a TCS-SP8 confocal laser scanning microscope.

3. Results and Discussion
The morphology and chemical composition of Se-CQDs TEM image of Se-CQDs is presented in Figure 2a. It can be seen that the average size of Se-CQDs is about 3-4 nm, which was consistent with the AFM characterization of Se-CQDs (Figure 2b), demonstrating the nanometer size and narrow size distribution of carbon quantum dots. XPS survey scan of Se-CQDs (Figure 2c) suggests that besides the elements of C, O, and N, an additional element of Se is appeared, proving the successful doping of Se element in Se-CQDs.

![Figure 2.](image)

Figure 2. (A) TEM image of Se-CQDs, scan bar is 20 nm. (B) AFM image of Se-CQDs. (C) XPS survey scan of Se-CQDs.

The fluorescence properties of Se-CQDs were then identified by UV-vis and fluorescence spectra (Figure 3). Figure 3A shows that the absorption peak of Se-CQDs is centered at 267 nm, corresponding to the π-π transition of graphitic carbon. The emission spectrum suggests that with the excitation of 267 nm, the emission peak of Se-CQDs is at about 440 nm, corresponding to blue fluorescence, as observed in the inset of Figure 3A. Figure 3B shows that with different excitation wavelength, the Se-CQDs exhibit different emission peak, demonstrating the excitation-dependent fluorescence. Moreover, it is found that the fluorescence intensity of Se-CQDs were nearly the same at different pH. In addition, the fluorescence intensity of Se-CQDs is not attenuated with illumination time, these results demonstrate the resistance of Se-CQDs to photo bleaching and good optical properties of Se-CQDs.

![Figure 3.](image)

Figure 3. (A) Absorption spectrum (a) and emission spectrum of Se-CQDs. (B) Emission spectra of Se-CQDs with different excitation wavelength. (C) FL intensity at different pH. (D) FL intensity of Se-CQDs illuminated with different times.
The detection of various kinds of ROS by Se-CQDs was then characterized, as shown in Figure 4. It is found that the fluorescence intensity of Se-CQDs increases with increasing concentrations of ·OH, \( \text{O}_2^- \), and \( \text{H}_2\text{O}_2 \). And the signal ratio shows good linearity with the concentration of ROS in the range of 0.1-50 μM, suggesting the Se-CQDs could be used for quantitative detection of various ROS. We have also carried out cytotoxicity test on Se-CQDs, the results (not shown here) indicate that Se-CQDs are compatible with live cells with cell viability of more than 90% even with high concentration of Se-CQDs of 60 μg/mL.

![Figure 4](image-url)

**Figure 4.** Effect of concentrations of (A, B) ·OH, (C, D) \( \text{O}_2^- \), and (E, F) \( \text{H}_2\text{O}_2 \) on the fluorescence intensity of Se-CQDs.

After demonstration of the ROS detection ability of Se-CQDs, we then modified the Se-CQDs with three kinds of molecules 1-3 that targeting organelle of mitochondria (with 1), lysosome (with 2), and endoplasmic reticulum (with 3). FR-IR spectra (not shown here) evidence the successful anchoring of targeting molecules on surface of Se-CQDs. To clarify the intracellular localization of modified Se-CQDs, we have carried out confocal fluorescence studies on modified Se-CQDs and commercial organelle marker incubated RAW264.7 cells (Figure 5). For example, Figure 5a displays the image of 1-modified Se-CQDs stained cells, while Figure 5b is the image of a commercial mitochondria marker-stained cells, it can be clearly seen that they are significantly overlapped with each other, as indicated by the bright pink signals in merged image (Figure 5c), with a high Pearson’s coefficient of more than 0.90 that obtained from the intensity correlation plots (Figure 5d). The 2-modified Se-CQDs in lysosome (Figure 5e-5h) and 3-modified Se-CQDs in endoplasmic reticulum (Figure 5i-5l) show similar results as those of 1-modified Se-CQDs in mitochondria, demonstrating the successful targeting detection of ROS by our modified Se-CQDs.

![Figure 5](image-url)

**Figure 5.** Localization studies of (a-d) 1-modified Se-CQDs in mitochondria, (e-h) 2-modified Se-CQDs in lysosome, and (i-l) 3-modified Se-CQDs in endoplasmic reticulum.
4. Conclusions
This paper develops novel targeting molecules modified Se-CQDs for targeting detection of ROS. The Se-CQDs are first synthesized by a facile hydrothermal process from green resource, and then modified by three kinds of targeting molecules via amidation reaction. The Se-CQDs exhibit good optical properties and quantitative response to various ROS, while the modified Se-CQDs show good targeting detection ability to different organelles. It is expected that our modified Se-CQDs could find wide application in targeting detection of ROS.

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