Uptake of water-soluble CdTe quantum dots in living cells of euglena gracilis

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Water-soluble CdTe quantum dots (QD600) were synthesized by hydrothermal route. The uptake of QD600 in living cells of euglena gracilis was studied. The luminescence spectra show that the QD600 were bound in cells after incubation. The fluorescence images, measured by confocal laser scanning microscope, demonstrate that the QD600 can penetrate into the cells. The amount of cell-bound QD600 increased with incubation time of QD600 at 25 °C, but no detectable QD600 were found in the cells when the incubation temperature was 4 °C, it was suggested that the QD600 were actively taken into the cells by endocytotic pathway. These results indicate that the water-soluble CdTe quantum dots have the potential in the application of bio-labeling.

OCIS codes: 040.5570, 260.3800, 000.1430.

Water-soluble CdTe quantum dots (QD600), as fluorescent semiconductor nanocrystall, have several unique optical and chemical features comparing with organic dyes, such as higher quantum yield, good photostability, and a narrow tunable emission spectrum. These features make them become desirable fluorescent material for bio-labeling[1]. Euglena gracilis, a unicellular green alga, demonstrated remarkable ability to transport heavy metal ions into the cell compartments[2]. Here the euglena gracilis was used as a living model to study their uptake to quantum dots as well as the uptake mechanism.

The preparation of 1-thioglycerol-capped CdTe particles has been described in detail elsewhere[3]. Briefly, 22 mL of freshly prepared oxygen-free 0.05–mol/L NaHTe solution was added to 125 mL of 0.013-mol/L nitrogen-saturated $Cd(ClO_4)_2 \cdot 6H_2O$ aqueous solution at pH 11.2 in the presence of 0.5 mL of 1-thioglycerol as a stabilizing agent. The solution was heated to 200 °C and refluxed for 8 h to promote the growth of CdTe nanoparticles. The method of size-selective precipitation was used to separate a CdTe nanoparticle fraction with relatively narrow (¡10% as confirmed by transmission electron microscopy) size distribution[4].

The euglena gracilis was procured from the Institute of Hydrobiology (Wuhan) of the Chinese Academy of Science, and maintained as previously described [5]. Aliquots from liquid stocks were added to a 250-mL flask containing 100-mL liquid medium. Culture was exposed to a 12-h light (2300 lx)/12-h dark cycle at 25 °C for 48–72 h. Aliquots of culture were centrifuged at 2000 r/min for 3 minutes at room temperature and re-suspended in deionized water to remove culture medium. These cells were then added with QD600 (4.5 μg/mL) for over night incubation. After incubation the cells were centrifuging-washed 3 times to remove un-bound QD600 and then the cell density was adjusted to 1.0×10^5 cell/mL for spectrum measurements. The luminescence spectrum of QD600 treated cells was measured with the spectrometer (F-2500, Hitachi).

Figure 1 shows the luminescence spectra of QD600 in water solution and cell-bound QD600 in cell suspension. The luminescence peak of cell-bound QD600 is red-shifted about 5 nm comparing to that in water solution, reflecting that QD600 may bind to some bio-molecules. The typical luminescence peak of cell suspension indicates that the QD600 was taken up by the cells after incubation.

The QD600 can penetrate into the living cells, its cellular distribution was studied by the confocal laser scanning microscopy (Olimpas, V300) then. The excitation was 488-nm laser from attached argon-krypton laser. The filter between 580 and 630 nm was used to capture the fluorescence images of QD600 in cells. The 60× objective in microscopy (Olimpas) was used in measurements. Due to the function of pinhole in machine, the good resolution of about 0.5 μm on Z-axis can be obtained. Thus the Qd600 intracellular distribution in euglena gracilis can be visualized from the fluorescence images obtained. The typical image of QD600 intracellular distribution was shown in Fig. 2, he excitation was 488 nm, and the band-pass filter (585–630 nm) was used for image acquiring. It can be seen that the QD600 really penetrates into the cells and distributes in the cytoplasm of cells with diffuse characteristic.

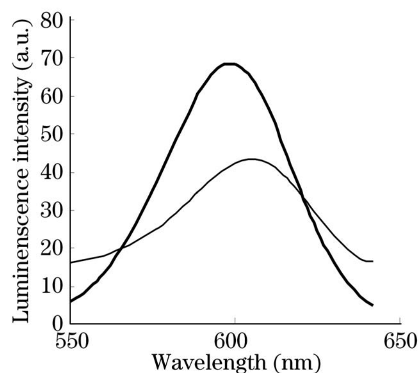

Fig. 1. The luminescence spectra of QD600 in euglena gracilis. The boldfaced curve is QD600 in the water and the thin curve is QD600 in living euglena gracilis.

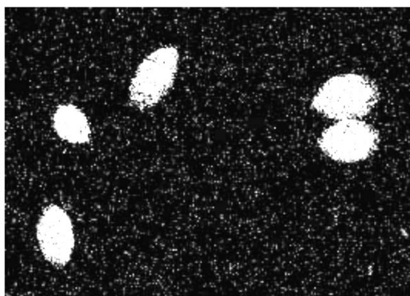

Fig. 2. The luminescence image of Qd600 in euglena gracilis.

The cell can take up the QD600, the uptake kinetics of cells was explored further. The cells were divided into different groups with the same cell density. The different cell groups were incubated with QD600 for different time. After incubation, the luminescence intensity of each cell sample was measured. The luminescence intensity of the cell sample is proportional to the cellular content of QD600. Thus, the uptake kinetics of cells could be acquired according to the relative luminescence intensities of different cell groups. Figure 3 demonstrates the uptake process of cells with the characteristic that the cellular content of QD600 increases with the incubation time at 25 °C.

The QD600 can penetrate into the cells. However, that in which way the QD600 passes through the outer membrane into the cells — diffusion or endocytosis? Diffusion is a passive physical process. The changing in temperature moderately affects the diffusing speed, but cannot stop the diffusion. Endocytosis is an active physiological function for living cells, which only takes place in physiological temperature. Thus a low temperature, which is below the physiological temperature, can be used in cell incubation of QD600 to distinguish the pathway of QD600 penetration.

Figure 3 shows the comparison results of cell incubation

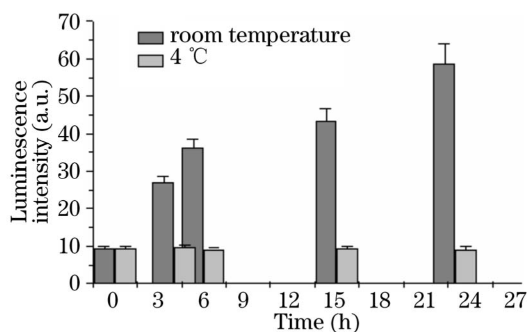

Fig. 3. The uptake kinetics of QD600 in euglena gracilis at 25 °C (room temperature) and 4 °C.

in low temperature (4 °C) and normal temperature (25 °C). It is demonstrated that there were no detectable cell-bound QD600 under low temperature incubation, even for longer time incubation. This result reveals that the endocytosis might be an important pathway in QD cellular uptake of euglena gracilis.

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