Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 139 (2015) 179–183

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Study of the interactivity between mercury and cellular system labeled with carboxymethyl chitosan-coated quantum dots and its application in a real-time in-situ detection of mercury

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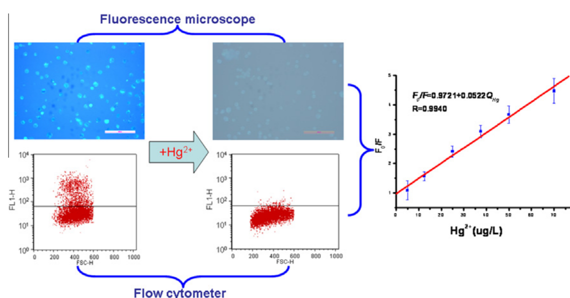
HIGHLIGHTS

- A fluorescent probe is synthesized using CMC-coated CdTe quantum dots.
- MDCK cells are fluorescently labeled by the probe to obtain a stable fluorescence.
- When Hg^{2+} interacts with the QD-cells, the fluorescence quenching process occurs.
- The effect can be quantitatively described by the Stern–Volmer equation.
- A method for real-time and in-situ detection of Hg in living cells is provided.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 4 December 2014
Accepted 12 December 2014
Available online 25 December 2014

Keywords:
Quantum dots
Carboxymethyl chitosan
Mercury
Cell

ABSTRACT

In this study, canine kidney cells (MDCK) are fluorescently labeled by carboxymethyl chitosan-coated CdTe quantum dots to obtain a stable fluorescence. Fluorescently labeled MDCK cells are incubated with Hg^{2+} and passed flow cytometer to measure the mean fluorescence intensity, which shows $[Hg^{2+}]$ has a prominent quenching ability on the cells' fluorescence. The dose-dependent relation can be described by Stern–Volmer equation at the concentration range of 5–70 μg/L $[Hg^{2+}]$. This method can be employed to determine the concentration of Hg^{2+} in living cells by measuring the changes in fluorescence of the cellular system. The results show a relative standard deviation of 7.16% ($n = 11$) and a recovery rate ranging from 92% to 103%, indicating a promising prospect of application on real-time in-situ analysis of $[Hg^{2+}]$ and its cytotoxic effects.

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Introduction

Mercury is one of the most dangerous and widespread pollutant which has attracted considerable concerns from environmental and medical sciences due to its toxic and enduring effect on human body. Environmental pool of mercury keeps growing as a result of heavy industrial activities. The mercury (II) ion and its derivatives can be absorbed biologically through diet and drinking water and progressively accumulate when the contaminants move up the food chain [1]. Therefore, analysis and measurement of trace mercury in environmental and biological samples is extremely important. Current techniques for mercury determination involve spectrophotometry, atomic absorption/emission spectroscopy, inductively coupled plasma-mass spectroscopy (ICP-MS), and

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http://dx.doi.org/10.1016/j.saa.2014.12.049
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inductively coupled plasma-atomic emission spectrometry (ICP-AES), etc. [2–5]. Although these methods have low limits of detection and wide linear ranges, they are hardly suitable for the detection of mercury in living cells. Since kidney is a main target of mercury toxicity in the body, we use canine kidney cell (MDCK) as a model system in this study to develop an in-situ, real-time detection method which can be applied in studying the renal distribution and dynamics of Hg^{2+} and in assisting the identification of molecular mechanisms underlying Hg^{2+}-induced nephrotoxicity.

Recently, the advantages of fluorescence signaling with its intrinsic sensitivity have encouraged the development of a series of fluorescent sensors for Hg^{2+}, which have proved to be a rapid and reliable method in tracking Hg^{2+} present in various kinds of samples [6–9]. However, these fluorescent dyes are subject to limitations such as low signal intensity and photo-bleaching. Furthermore, most of these fluorophores have narrow excitation spectra and often exhibit broad emission bands with red tailing. Quantum dots (QDs) and semiconductor nanocrystals have attracted considerable attention in various fields of biological and medical research. Due to their strong fluorescence, broad excitation range, narrow emission spectra and high photostability, QDs seem to be ideal fluorescent tags for long-term imaging of whole cells or intracellular molecules and structures [10,11].

In a previously published paper, we described a method that we developed to synthesize CdTe QDs in aqueous phase with microwave irradiation under controlled temperatures [12]. In order to reduce QDs' cytotoxicity and thus enabling their application in living cells, fluorescence probes were assembled by mixing CMC with QDs in the aqueous phase to form CMC-coated CdTe QDs (CMC-QDs). In general, MDCK cells were first optimally conditioned and then labeled with prepared fluorescent probes. Mercury was introduced into the micro-system to examine its quenching effects. Using a dynamic quenching model, a dose-dependent relationship was established between the concentration of Hg^{2+} and the fluorescence change, allowing a real-time in-situ analysis of exogenous Hg^{2+} in living cells.

Materials and methods

Instrumentation

A Milestone microwave digestion/extraction system equipped with a temperature controller was used for the preparation of alloyed CdTe QDs. The cells were cultured in a CO_2 controlled incubator (U.S. SHELLAB 2325-2), Cell imaging was recorded with a fluorescence microscope (Nikon 80i, Japan). The average intensity of cell fluorescence was measured using a FACSCalibur flow cytometer (Becton, Dickinson and Company, USA).

Reagents and materials

Dulbecco's Modified Eagle's Medium (DMEM) nutrient solution, fetal bovine serum, EDTA-trypsin, PBS buffer solution (pH = 7.2) were purchased from U.S. CIBCO. MDCK cell lines were provided by the Chinese Center for Disease Control and prevention; mercury standard solution (100 mg/L, the Chinese national standard material center).

Synthesis of CdTe/CdS QDs by microwave irradiation and preparation of CMC-QDs fluorescent probes

Details of the QD and CdTe precursors preparation was described elsewhere [12]. Briefly, Cd^{2+} was reacted with potassium hydride tellurium (KHTe) solution in conditions as previously reported [12]. KHTe solution was produced by reacting potassium borohydride (KBH_4) with tellurium powder at 0 °C or below. Then, 0.15 mmol TGA was injected into nitrogen-saturated 1.25 mM $CdCl_2$ aqueous solution (100 mL), as a stabilizer. The pH value of the CdTe precursors with a concentration of 1.25 mM was adjusted to 11.4 using 1 M KOH. Finally, the resulting CdTe precursors were transferred to a Teflon inner vessel placed inside a microwave digestion furnace, and heated by microwave irradiation under 100 °C for 15 min to obtain CdTe QDs.

Carboxymethyl chitosan and CdTe QDs were mixed in a 2:1 mass ratio, vigorously stirred and incubated at room temperature for 1 h. CMC-QDs fluorescent probes were generated by chelation between amido and carboxyl groups in CMC with QDs.

MDCK cells labeling with CMC-QDs

MDCK cells in logarithmic phase with good growing status were plated in a 35 mm glass-bottomed Petri dish at a density of 5×10^4 cells/dish and grown in DMEM overnight at 37 °C in 5% humidified CO_2. The cells were labeled with 0.2 ml of CMC-QDs solution (1 mM, filtered through a 0.22 micron filter head to remove bacteria before use) and 1.8 ml DMEM (final CMC-QD concentration 0.1 mM) at 37 °C in 5% CO_2 for 24 h. Periodically small aliquots of MDCK cells were checked to assure a successful labeling with fluorescence.

Interactivity between Hg^{2+} and cells labeled with CMC-QDs

After the labeling of MDCK cells, DMEM medium was discarded and the cells were washed twice with PBS to remove unbounded CMC-CdTe quantum dots. DMEM containing different concentrations of Hg^{2+} is added to the cells for incubation and the media was gently removed. Cells were then digested with EDTA-trypsin and re-suspended in 0.9 g/mL NaCl solution (1 mL for each dish). The mixture was transferred to a 5 mL test tube and centrifugated at 2000 rev/min for 5 min. After discarding the supernatant, 0.9 g/mL NaCl solution was added to resuspend the cells. Cells were washed twice, counted and diluted to an appropriate concentration for flow cytometry analysis.

Results and discussion

Effect of exogenous Hg^{2+} on unlabeled MDCK cells

Kidney is one of the main targets of mercury toxicity. It has been shown that under Hg^{2+} treatment, H_2O_2 level in renal tissues significantly increases, accompanied by excessive generation of different types of reactive oxygen species, reduced intrarenal content of glutathione (GSH) – an important antioxidants, and suppressed renal superoxide dismutase (SOD) activity, suggesting that oxidative stress is an important mechanism for mercury-induced acute renal injury [13,14]. Mercury ions actively form complex with sulfhydryl groups, thus compromising the activity of cellular enzymes such as cytochrome oxidase, pyruvate kinase, succinate dehydrogenase etc., which play vital part in cellular metabolism. Mercury treated cells show impeded metabolism and abnormal energetics which eventually leads to cell degeneration and death.

In order to better understand the interactions involved in the ternary system of CMC-QDs, MDCK cells, and Hg^{2+}, we first examined the effects of Hg^{2+} on unlabeled MDCK cells. Different amount of Hg^{2+} was incubated with MDCK cells and cell morphology was monitored under inverted microscope on a daily basis. The mean fluorescence intensity of the system was measured by flow cytometry after a 3-day incubation. The results suggest that in the range 5–100 μg/L, Hg^{2+} treatment does not affect auto-fluorescence of the cell and shows minimal effect on cell growth.

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