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Copper clusters-based luminescence assay for tetracycline and cellular imaging studies

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ABSTRACT

Two copper nanoclusters (NCs) stabilized by L-cysteine and glutathione have been reported. The nanoparticles with ultrafine size had excellent water solubility and exhibited green or red emission respectively. Particularly, the two copper clusters were found to be responsive to the presence of tetracycline (TC) and *on-off* quenching effects were achieved. After incubation, the cell staining and target-detection features could be still effective in two adherent live cells. The nanocluster showed very low cytotoxicity and excellent biological compatibility. Accordingly, the special photophysical properties of copper NCs will be attractive for various potential applications such as bio-sensing and medical diagnosis.

1. Introduction

The discovery of metal nanoclusters (NCs) has aroused considerable interests due to their unique photoelectrical properties and biological compatibility [1–3]. These nanosystems generally possess narrow size distributions. They maintain the fundamental properties of materials from larger nanoparticles to the molecular scale entities. In particular, they have a wide range of application in numerous fields, such as optical sensing, analysis, bioimaging, and so on [4–6]. Owing to the extremely small size, better quantum efficiency, good photostability, biocompatibility, water solubility and low toxicity, the NCs are suitable for biological analysis and medical diagnosis [7–10]. Up to now, gold and silver nanoclusters have been fully developed [11,12]. But we should not underestimate the influence of copper particles on the sensing fields since the low-cost copper element is earth-abundant compared with noble metals. Currently, a few template preparation methods such as thiols, peptides, proteins and dendrimers were reported [13–15]. With the aim of improving its compatibility, the novel synthesis by using the biomolecule as the template or scaffold has induced much attention. Very recently, the tannic acid attached Cu NCs as the Fe^{3+} sensor has been reported [16]. L-cysteine capped CuNCs was employed for the assay of Hg^{2+} [17]. The bovine serum albumin stabilized CuNCs has been developed for the determination of kojic acid (KA) with good sensitivity and selectivity [18]. In this case, the studies of simple and economic techniques to achieve the optically active CuNCs for the detection of various targets have been paid much attention.

As the important member of widely-used antibiotics, tetracycline (TC) has been explored for the treatment of many bacterial infections and animal breeding industry as a growth agents [19]. Owing to its multiple antibacterial properties, excellent therapeutic effect and low cost, TC has been extensively applied. However, excessive amounts of TC can cause bacterial resistance and accumulation of antibiotics in soil, waste water and animal products. In addition, the unexpected residues of the TC in the environment would lead to suppression of microorganism growth. It is harmful to ecotype and poses a serious threat to human health [20]. Thus, it is necessary to develop a facile, rapid and reliable quantification method to monitor TC residues. Enzyme linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC) and electro-chemoluminescence have been reported [21–23]. However, these techniques usually require complicated processes and the use of expensive equipment. In recent years, fluorescent detection has become a powerful approach in analytical fields, owing to its advantages of convenient operation, low cost, real-time detection and high sensitivity.

In this work, we have assembled two kinds of Cu NCs by using both

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the L-cysteine and glutathione as the precursors. The above two functional scaffolds are readily available from commercial sources. The obtained nanoparticles with water solubility and excellent optical performance have been studied by UV–vis absorption, infrared spectra, photoluminescence, microscope and Energy Dispersive Spectrometer (EDS). More importantly, the difference between the band maxima of the excitation and emission for the two synthetic Cu NCs were relatively large. The corresponding Stokes shifts were measured to be 125 nm (Cu NCs-1) and 222 nm (Cu NCs-2) respectively. The large Stokes shifts will reduce the possibility of re-absorption and improve signal quality. Furthermore, experimental results demonstrated the cellular entry and application of Cu NCs-1with green emission in two adherent cell lines imaging. In the sensing experiments, TC has a significant effect on the emission of Cu NCs. This phenomenon could be observed by naked eye under the irradiation of UV-light (365 nm). The green and red emissions of the two probes enable the quantitative assay of TC in the micromolar range with high selectivity. Therefore the Cu NCs could be promising for the design of a TC sensor. Furthermore, we also incorporated the Cu NCs into the cellular environments. The entry of the green probe in two live cell lines (Hela and 697) demonstrated the potentials of this sensor in cell imaging.

2. Experimental

2.1. Materials

Copper (II) sulfate penta-hydrate ($CuSO_4{\cdot}5H_2O$), L-cysteine, glutathione, Sodium hydroxide (NaOH) and tetracycline hydrochloride were purchased from J & K company. The RPMI1640, DMEM,PBS buffer and other cell culture reagent were purchased from Sigma-Aldrich. Cell Titer 96® AQueous One Solution Cell Proliferation Assay (MTS) was provided by Promega. Milli-Q water (18.25 MΩ cm at 25 °C) was used throughout the experiment. All the other reagents were provided by Guangzhou Chemical Reagent Factory and used without further purification.

2.2. Characterization

Photoluminescence spectra were measured by a computer controlled HITACHI F-4600 fluorescence spectrophotometer. Absolute quantum yields were determined at room temperature through an integrating sphere (Edinburgh FLS 920 spectrometer). FT-IR spectra were measured using a Shimadzu Prestige. UV–vis spectra were recorded on an Agilent 8453 spectrophotometer. Lifetimes were collected by an Edinburgh FLS920 spectrometer. Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100HR. Size distribution has been determined by a dynamic light scattering instrument (Autosizer 4700, Malvern Instruments, UK). Confocal microscopy images were taken with a Zeiss confocal laser scanning microscope (LSM710) equipped with a laser at 405 nm. The MTS was measured at 490 nm using a Polarstar microplate reader. Flow cytometric analysis was performed on the BD Accuri C6 Flow Cytometer. The pH dependence experiments were carried out as follows: 50 μl of Cu NCs solution and 50 μl of water were added into 900 μl of 0.1 M phosphate buffered saline (PBS) solution at different pH values and the mixture was equilibrated for 10 min before spectral measurement. The fluorescence intensity at 493 nm was recorded using an excitation wavelength of 368 nm.

2.3. Synthesis of Cu NCs

Cu NCs were prepared by using L-cysteine and glutathione according to the references [17,24]. In order to describe the information clearly, the Cu NCs capped with L-cysteine was named as Cu NCs-1. The other one was called Cu NCs-2.

The Cu NCs-1 was obtained as follows: 1 mL of 10 mM $CuSO_4$ and 2.0 mL of 10 mM L-cysteine solution were added into 7 mL of deionized water under vigorous stirring. After 5 min, 0.3 mL of 1 M NaOH was dropped into this aqueous solution under vigorous stirring (pH ~ 7.0). The solution has been stirred for two hours at room temperature and Cu NCs-1 has been achieved. In order to remove the residues, the resulting transparent solution was further dialyzed for 24 h with a 1000 MWCO dialysis membrane. Finally, the purified Cu NCs-1 aqueous solution was stored at 4 °C for future measurements.

The synthesis steps of Cu NCs −2 were described here: firstly, 5.0 mL of GSH solution (50 mg/mL) was added into 5 mL of $CuSO_4$ solution (10 mM) under vigorous stirring. Five minutes later, 0.35 mL of NaOH solution (1 M) was added dropwise until the turbid liquid turned to transparent light yellow. The corresponding pH value was controlled to 7.0. Subsequently, the mixture solution was stirred vigorously at 37 °C for1h. Finally, the obtained product was dialyzed for 24 h with a 1000 MWCO dialysis membrane to remove the unreacted reagents. The purified Cu NCs-2 was also stored at 4 °C. The possible formation mechanism has been described as follows: the metal-ligand interactions refer to the copper (II) ions coordination and stabilization by thiol moieties as well as carboxylate groups in glutathione. Accordingly, GSH has been employed as both the stabilizing reagent and the reductant. The incorporation of NaOH would increase the reducing capability of thiol units. In this way, Cu^{2+} ion has been transformed to Cu atom and CuNCs have been generated. The CuNCs growth and formation could be monitored by the color change from colorless to light green. The reaction carried out at lower temperature (such as room temperature) was slow. It has been found that temperature elevation (37 °C) would speed up the reaction within relatively short time (around one hour).

2.3.1. Cell culture and apoptosis analysis

Hela cells were cultured in Dulbecco's modified medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. Cells were maintained at 37 °C in 5% CO_2. The 697 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) and maintained at 37 °C with 5% CO_2. To investigate apoptosis analysis, untreated cells and cells treated with Cu NCs were both stained with Annexin V-APC/7-AAD. Hela and 697 cells (10^6 cells/well) were dispersed within replicated 12-well microtiter plates and incubated for 24 h at 37 °C with 5% CO_2. After removal of the medium, cells were incubated with fresh medium (DMEM or RPMI 1640, 10% FBS and 1% PS) containing Cu NCs (0 and 100 μg/mL) and Cu^{2+} (100 μg/mL) for another 24 h. The system needs to be washed with bonding buffer twice and requires centrifuging. 100 μl buffer and 3 μl of 7-AAD and Annexin V-APC were added to each sample. Samples were incubated for 15 min and analyzed by flow cytometry on a BD Accuri C6 Flow Cytometer.

2.4. MTS assay

Two cell lines (Hela and 697) were used in this study. Hela cells and 697 cells were cultured in DMEM or RPMI 1640 media with 10% FBS and 1% antibiotics penicillin and maintained at 37 °C with 5% CO_2, respectively. Both of the two cell lines within replicate 96-well plates were pre-cultured at standard culture condition for one day. After removal of the medium, cells were incubated with fresh media containing Cu NCs (0, 5, 10, 20, 50, and 100 μg/mL), Cu^{2+} (100 μg/mL) for another 24 h. Then 3 μl MTS solution was added to each well. After incubation with 2 h, the absorbance was measured at 490 nm using a Polarstar microplate reader.

2.5. Cell incubation and imaging

The two cell lines were incubated at standard culture conditions (as mentioned above) for one day. In the experiments, cells were washed with media, incubated in the fresh media containing Cu NCs (100 μg/