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Copper nanoclusters as a highly sensitive and selective fluorescence sensor for ferric ions in serum and living cells by imaging

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ABSTRACT

A simple, one-step facile route for preparation of water soluble and fluorescent Cu nanoclusters (NCs) stabilized by tannic acid (TA) is described. The as-prepared TA capped Cu NCs (TA-Cu NCs) are characterized by UV–vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, luminescence, transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy (XPS). The TA-Cu NCs show luminescence properties having excitation and emission maxima at 360 nm and 430 nm, respectively, with a quantum yield of about 14%. The TA-Cu NCs are very stable even in 0.3 M NaCl, and their luminescent properties show pH independent. The fluorescence (FL) of Cu NCs is strongly quenched by Fe^{3+} through an electron transfer mechanism, but not by other metal ions. Furthermore, the FL of the TA-Cu NCs shows no changes with the addition of Fe^{2+} or H_2O_2 individually. On this basis, a facile chemosensor was developed for rapid, reliable, sensitive, and selective sensing of Fe^{3+} ions with detection limit as low as 10 nM and a dynamic range from 10 nM to 10 μM. The proposed sensor was successfully used for the determination of iron contents in serum samples. Importantly, the Cu NCs-based FL probe showed long-term stability, good biocompatibility and very low cytotoxicity. It was successfully used for imaging ferric ions in living cells, suggesting the potential application of Cu NCs fluorescent probe in clinical analysis and cell imaging.

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1. Introduction

Iron ions play crucial roles in biological systems because ferrous/ferric (Fe^{2+}/Fe^{3+}) is one of the important redox pairs for electron transport in the respiratory chain and for a variety of enzymatic reaction in biological systems (Que et al., 2008). The unbalance of iron in human body leads to many diseases (Omara and Blakley, 1993; Allen, 2002). Thus the determination of iron ions in the biological system is very significant. Various analytical methods have been developed for determination of iron ions, including flame atomic absorption spectroscopy (FAAS) (Ajlec and Stupar, 1989), voltammetry (Van den Berg, 2005), colorimetric method (Luan and Burgos, 2012), chemiluminescence (Bowie et al., 2002), and fluorescence detection (Dwivedi et al., 2011; Ho et al., 2012; Bricks et al., 2005).

Due to its high sensitivity, the fluorescent probes have been recognized as the efficient molecular tools to help monitor and visualize trace amounts of samples in live cells or tissues. Recently, nanoclusters (NCs) are becoming a focus of considerable interest and one of very promising fluorescent probes owing to their special advantages, such as low toxicity, high photoluminescence yield and good biocompatibility, which made them potential applications in single-molecule imaging, biolabeling, sensing, *etc.* (Schaeffer et al., 2008; Lu and Chen, 2012). However, the previous studies concentrated much effort on gold sub-nanometer clusters. Only in recent years, Ag and Cu NCs have attracted considerable interest due to their unique photoluminescent properties and thus the potential applications in fluorescence analysis (Lu and Chen, 2012). For example, a few studies have been manipulated on fluorescence Cu NCs, using some surface protecting ligands such as DNA (Rotaru et al., 2010; Chen et al., 2012; Zhou et al., 2011), proteins (Goswami et al., 2011), polymers (Kawasaki et al., 2011), and thiols (Yuan et al., 2011). These surface protecting ligands improve their stability and water solubility by providing a protecting layer on the surface of Cu NCs. However, these macromolecular ligands resulted in the formation of Cu NCs with large hydrodynamic radius, which limited the scope of potential applications (Adhikari and Banerjee, 2010; Bao et al., 2010). Most recently, benzotriazole was applied as a template to reach desired smaller size (Salorinne et al., 2012). However, relatively complicated

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synthetic procedure and constrained accessibility to the materials greatly limited applications of the as-prepared Cu NCs. Furthermore, careful attention needs to be paid to the synthesis of tiny Cu clusters due to their easy oxidation. Thus, development of a simple and facile strategy for the synthesis of water soluble, extremely stable, and highly quantum efficient fluorescent NCs is highly valuable, but even more challenging.

Herein, for the first time, we reported the successful synthesis of Cu NCs stabilized by tannic acid using one-pot method in which $CuSO_4$ was reduced by ascorbic acid. The as-prepared Cu NCs exhibited the blue emission at 430 nm with a high quantum yield (QY) of 14%. It was found that the fluorescence (FL) of Cu NCs is strongly quenched with the addition of Fe^{3+} through specific binding of Fe^{3+} and tannic acid to form a complex (Ernst and Menashi, 1963). More importantly, H_2O_2 and other common metal ions, including Fe^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cr^{3+}, In^{3+}, Na^{+}, and K^{+}, have minor effects on the fluorescence of Cu NCs. This indicates that the as-prepared Cu NCs show high selectivity toward Fe^{3+}. Based on this new finding, a facile, green, sensitive, and selective chemosensor was developed to detect Fe^{3+} in serum samples and image Fe^{3+} in living cells, showing great potential in biological applications.

2. Materials and method

2.1. Materials

All chemicals and reagents were of analytical grade and used as received without further purification. Tannic acid (denoted TA) was purchased from Qiangshun Chemical Reagent Co., Ltd. (Shanghai, China). $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ were purchased from Signopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ascorbic acid was purchased from Chengdu Kelong Chemistry Reagent Factory (Sichuan, China). $CuSO_4$, NaOH, HCl and citrate acid were obtained from Chongqing Chemical Reagent Company (Chongqing, China). The A549 cell used in the study was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Ultra-pure water was prepared in the lab using a water treatment device.

2.2. Instrumentation

The fluorescence spectrum and intensity were recorded on a Hitachi F-7000 Fluorescence spectrophotometer (Tokyo, Japan). The pH of the solutions was measured by a PHS-3D pH meter (Shanghai Precision Scientific Instruments Co., Ltd., China). The UV–visible spectra were measured on a Hitachi U-4100 spectrophotometer (Tokyo, Japan). Dynamic light scattering (DLS) was performed on a Zetasizer Nano-ZS90 (Malvern, UK) instrument for characterization of the size distribution of the Cu NCs in a solution. X-ray photoelectron spectroscopy (XPS) spectra were measured by a XSAM-800 X-ray photoelectron spectrometer (Kratos, UK). The morphology of Cu NCs was observed using transmission electron microscopy (TEM, LIBRA 200, ZEISS) at an acceleration voltage of 200 kV. Cellular images were obtained with an Olympus IX70 inverted fluorescence microscope system, equipped with a 100-W mercury lamp, 40 × objective (Olympus) and a U-MWU filter set (330–385/400/420 nm, Olympus, Japan).

2.3. Preparation of copper nanoclusters

In a typical preparation process, a solution of $CuSO_4$ (0.2 mL, 0.1 M) and tannic acid (0.1 mL, 1 mM) in water (20 mL) was stirred at room temperature for 5 min. After ascorbic acid (0.2 mL, 1 M) was added to the mixture, the resulting pale blue mixture solution was stirred for 6 h at 50 °C to obtain yellowish Cu NCs. A dialysis membrane (MWCO: 3500 Da; pore size: ca. 0.35 nm) was then used to separate the Cu NCs from any residual un-reacted species. The as-prepared TA stabilized Cu NCs (denoted TA-Cu NCs) were stored in a refrigerator at 4 °C until use.

2.4. General procedure for fluorescent detection

In a typical process, a series of working standard Fe^{3+} solutions with different concentrations were added to TA-Cu NCs solution (final concentration of 3 μM). The mixture was mixed well, and then FL spectra measurements and photographs were taken. The FL signal was monitored by a photomultiplier tube (PMT, operated at 700 V) of the Type F-7000 Fluorescence spectrophotometer and was recorded by a computer. The relative fluorescence intensity $[(I_0 - I)/I_0]$ *versus* Fe^{3+} concentration was used for calibration. Here, I_0 and I are the fluorescence intensities of the TA-Cu NCs before and after adding analytes, respectively. At every Fe^{3+} concentration, the measurement was repeated thrice, and the average FL signal was obtained.

2.5. Procedure for the determination of serum iron

Pretreatment of serum samples was adapted from Kyaw (1976), with minor modification. Briefly, 2.0 mL of water was added into the 1.0 mL fresh serum, and then 2.0 mL of 20% trichloroacetic acid solution was added. The mixture was mixed well and put into an oscillator with continuous shaking for 45 min, then centrifuged at 2500 rpm for 15 min. The supernatant was collected and 20 μL of 3% H_2O_2 was added to 4.0 mL of supernatant to oxidize the Fe^{2+} ion to Fe^{3+} ion for further analysis. The fluorescent determination of serum sample was performed by the same way as Fe^{3+} standard except that Fe^{3+} solution was replaced by pretreated serum samples.

2.6. Cell viability assay

The cytotoxicity of TA-Cu NCs was examined by the cell-counting kit-8 (CCK-8, Dojindo Laboratories, Japan) assay (Yu et al., 2014; Wang et al., 2013). First, A549 cells were seeded in 96-well plates at a density of 3.25×10^4 cells mL^{-1}. After 24 h incubation, the medium was then replaced by the medium containing TA-Cu NCs with various concentrations (0, 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, and 10 μM), and the cells were incubated for another 24 h. Next, the cells were washed thrice with phosphate buffered saline (PBS), and freshly prepared CCK-8 (10 μL) solution in culture medium (90 μL) was added to each well. After 1 to 4 h incubation, the CCK-8 medium solution was carefully removed. Then, the plate was gently shaken for 10 min at room temperature, and the optical density (OD) of the mixture was measured at 450 nm. The cell viability was assessed by the following equation:

$$\text{Cell viability (\%)} = (\text{Sample OD} - \text{Blank OD})/(\text{Control OD} - \text{Blank OD}) \times 100\%$$

2.7. Cell imaging

The A549 cells were grown in modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2. Firstly, the cells were washed with PBS, followed by incubating with 0.2 mM TA-Cu NCs in 320 μL of medium for 5 h at 37 °C, and then washing with PBS five times. To detect Fe^{3+} in living cells, the cells were incubated with 0.2 mM TA-Cu NCs for 5 h and then stimulated with 0.05 mM Fe^{3+} and 0.1 mM Fe^{3+} for 1 h,

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