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A specific nanoprobe for cysteine based on nitrogen-rich fluorescent quantum dots combined with Cu^{2+}

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ABSTRACT

As a new member of the carbon quantum-dot family, fluorescent nitrogen-rich quantum dots (NRQDs) were prepared by a mixed solvothermal method using 2-azidoimidazole and aqueous ammonia as reactants. These NRQDs are rich in nitrogen up to 40.2%, which are endowed with high fluorescence quantum yield, good photostability, water-solubility and favourable biocompatibility. We further explored the use of NRQDs combined with Cu^{2+} as a nanoprobe for sensing fluorescently of cysteine (Cys) in complex biological samples. In this sensing system, the fluorescence is significantly quenched via energy transfer from NRQDs to Cu^{2+} for the coordination of amino-containing groups with Cu^{2+}. The strong affinity between Cu^{2+} and Cys leads to the formation of Cu^{2+}-Cys complexes and cause the detachment of Cu^{2+} from the surface of NRQDs, thus the fluorescence of NRQDs recover. This nanoprobe allows analysis of Cys by modulating the switch of the fluorescence of NRQDs with a detection limit of 5.3 nM. As expected, the proposed NRQDs-Cu^{2+}complex-based nanoprobes were successfully applied for the determination of Cys in human serum and plasma samples with recoveries ranging from 97.2% to 105.7%. The probe ensemble was also successfully applied to imaging of Cys in living cells with satisfactory results, which shows strong potential for clinical diagnosis.

1. Introduction

Fluorescent nanoparticles (NPs) including quantum dots have attracted considerable attention owing to their outstanding chemical selectivity, optical and electronic properties (Sarkar et al., 2015; Arul et al., 2017). Among the nanostructures, carbon quantum dots (CQDs) have emerged and attracted growing interest in analytical and bioanalytical chemistry in recent years (Atchudan et al., 2016; Borse et al., 2017). Compared with conventional semiconductor quantum dots (SQDs) that usually contain heavy metal, for example, Cd^{2+}, the good photostability, high biocompatibility, and low cytotoxicity make CQDs good alternatives to SQDs for drug delivery to biological and biomedical imaging in vitro and in vivo (Babakhanian. et al., 2015; Boyaci. et al., 2015). However, the CQDs, as a new type of fluorescent probe, did not show obvious advantages over small molecular dye or other nanostructure-based probes in terms of sensitivity and selectivity, mainly due to the fact that the conventional CQDs did not show satisfied fluorescence activities, leading to low detection sensitivities, or the CQDs were not well functionalized, resulting in bad selectivities (Caputo et al., 2017; Tian et al., 2013). Evidently, the development of specially functionalized CQDs-based probes with relatively high quantum yield, water solubility and favourable biocompatibility has become very important for the analytical applications.

Modification with coating materials may produce strong fluorescent CQDs–based probes with controllable biocompatibility and stability. Up until now, many methods have been developed for the modification of CQDs are mainly focusing on the optoelectronic devices and bioimaging (Ooi et al., 2007; Qing et al., 2014; Qian et al., 2015). CQDs have been endowed with molecular recognition ability through surface functionalization and applied in chemical and biologic sensing. Various coating materials such as polyamine, N-(2-aminoethyl)-N,N,N′-tris(pyridine-2-ylmethyl)ethane-1,2-diamine, polyamide, ethylenediamine and polyethyleneimine are frequently used to modulate the chemical, optical and electronic properties of CQDs, which make the modified CQDs-based probes sensitive and selective to detection and imaging the metal ions, for example, Cu^{2+} in live cells (Qu et al., 2012; Wang et al., 2017; Sarkar et al., 2014).

Doping with heteroatoms is another good strategy to tune the intrinsic properties of CQDs (Gao et al., 2016; Wu et al., 2016a, 2016b). As far as doping elements are concerned, electron-rich N atom is the most frequently used one to obtain the exceptional properties of CQDs (Kang et al., 2017; Weerapana et al., 2010a, 2010b). it has been shown that the modified CQDs with N could drastically increase quantum yield and give more active sites due to the electron-withdrawing ability of nitrogen atoms (Li et al., 2012; Niu et al., 2016;

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Stachniuk et al., 2016). To incorporate nitrogen groups into CQDs, several approaches have been developed, such as treatment of carbon materials with ammonia at high temperatures (Przepiorsi et al., 2004), carbonization of nitrogen containing compounds at high temperature (NCCs)(Jurgens et al., 2003), liquid phase polymerization of NCCs (Lee et al., 2009) or chemical vapor deposition with NCCs as a N source (Jin et al., 2011). However, all the above-mentioned methods suffer from a common drawback is that most of the nitrogen atoms were introduced into nanostructures under harsh conditions with relatively less content of element nitrogen (Hou et al., 2014; Zhu et al., 2009), which may limit their wide applications. Thus, developing simple and low-cost methods for the production of CQDs material with relatively abundant content of element nitrogen is highly desired.

Recently, nitrogen-rich quantum dots (NRQDs), as a new member of the carbon quantum-dot family were prepared by refluxing in methanol, which showed distinct and unique optical properties (Chen et al., 2014). Compared to popular CQDs modified with N, these NRQDs contain a higher percentage of nitrogen content and can effectively tune their chemical selectivity and optical properties. Preliminary application studies on biocompatible staining, determination of natural drug, and detection of toxic cation of NRQDs were also carried out. These results demonstrated that the NRQDs exhibit excellent photoluminescence, high photostability, high quantum yield, water solubility and favourable biocompatibility, which is extremely important for their practical applications (Wu et al., 2016a, 2016b; Carrillo-Carrio´n et al., 2009; Tang et al., 2017; Rani and John, 2016).

As one of the most important small biothiols, cysteine (Cys) plays versatile roles in a variety of physiological processes such as biocatalysis, providing resistance to the body against harmful effects, post-translation modifying, stabilizing protein structures and function (Seshadri et al., 2002; Wang and Cynader, 2001). In human, clinically abnormal level of Cys is associated with edema, liver damage, Alzheimer's disease, Parkinson's disease and other health problems (Weerapana et al., 2010a, 2010b; Refsum et al., 2004). The detection of Cys both in academic research and in clinical applications is important. Thus, we can expect that the above-mentioned excellent properties of NRQDs may helps in selective and sensitive detection of Cys in living cells.

In this work, we present a facile approach to synthesize fluorescent NRQDs using 2-azidoimidazole and aqueous ammonia as reactants by a mixed solvothermal method. The nitrogen content of the NRQDs is as high as 40.2%, which make it exhibit a good biocompatibility, water solubility and high fluorescence quantum yield. In cooperation with Cu^{2+}, a nanoprobe for sensing of Cys based on the NRQDs was constructed. The coordination of NRQDs with Cu^{2+} result in the emission quenching of NRQDs, while the addition of Cys can lead to the fluorescence recovery. On the basis of the fluorescence change, we developed a facile specific nanoprobe for sensing of Cys in human serum and plasma with flexibility. Especially, owing to the unique properties of the NRQDs-Cu^{2+} complex-based nanoprobe with good membrane permeability and excellent biocompatibility, it was further applied to imaging of Cys in human lung adenocarcinoma cells with a high selectivity.

2. Material and methods

2.1. Chemicals and instruments

All the reagents and chemicals were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China) and were used as received. Human serum, plasma samples and A549 cells were provided by XiangYa School of Medicine, Central South University (Changsha, China). UV–Vis absorption spectra were recorded on a UV–Vis 2600 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were carried out on a F-7000 Spectrofluorometer(Shimadzu, Japan). ^{1}HNMR spectra were recorded with the Bruker AVB-400 MHz NMR spectrometer (Bruker biospin, Switzerland). Transmission electron microscopic (TEM) images were recorded with a PHILIPS TECNAI 10 TEM instrument (Philips, Netherlands). Fluorescence imaging of A549 cells was examined by confocal laser scanning microscopy (Zeiss LSM 710NLO, Germany). The pH measurements were performed with a pH-meter PB-10 (Sartorius, Germany).

2.2. Synthesis of NRQDs

Fluorescent NRQDs were synthesized by a mixed solvothermal method. As a required intermediate for the preparation of NRQDs, 2-azidoimidazole was prepared from 2-aminoimidazole, NaN_3, and $NaNO_2$ in aqueous HCl solution (Xue et al., 2015). Then the raw NRQDs were synthesized by a toluene/water-based solvethermal method using 2-azidoimidazole (1.10 g), benzyltrimethyl ammonium bromide(0.001 g), toluene(28 mL) and aqueous ammonia (7 mL, 25% in water) added into the high pressure reactor. The reaction mixture was warmed to 115 °C and react for 2 h. The resulting solution finally turned greenish black and even to dark brown, which implied the formation of NRQDs. After centrifugation at 12,000 rpm for 25 min, the supernatant was collected and then dialyzing against ultrapure water through a dialysis membrane(7.0 KD) for 36 h. The dialysate was dried under vacuum-freeze to give an cinnamon-colored solid residue.

2.3. Construction of a fluorescent nanoprobe for Cys combined NRQDs with Cu^{2+}

An appropriate amount of NRQDs solution (10.0 mL, 100 μg/mL) and 2.5 mL of Cu^{2+} (5.5 nmol/L) solution were added to a 20 mL cuvette, then incubated for 10 min for preparation the NRQDs-Cu^{2+} nanoprobe. Fluorescence quenching of NRQDs was used to monitor the formation of NRQDs-Cu^{2+} nanoprobe.

2.4. Detection of Cys with the NRQDs-Cu^{2+}-based nanoprobe

The prepared NRQDs-Cu^{2+} nanoprobes and appropriate aliquot of Cys solution were transferred into a cuvette and incubated for 10 min at room temperature. Then the fluorescence intensity of the mixture was recorded at 472 nm (*Ex* = 400 nm), and the slit widths of emission and excitation were fixed at 5 nm. The detection procedures for other interferences including amino acid and biothiols were similar to Cys. When real samples were detected, Cys standard solution was substituted by the human serum and plasma solutions. Each determination is repeated three times.

2.5. Intracellular Cys fluorescence imaging in A549 cells

To examine whether the NRQDs-Cu^{2+} nanoprobes can sense Cys in living cells, we then detected the Cys in A549 cells treated by the nanoprobes. Two days before fluorescence imaging, A549 cells were seeded in four plates containing sterile petridish and were incubated in a DMEM supplemented solution with the addition of 15% (v/v) fetal bovine serum (FBS), penicillin (80 U/mL), and streptomycin (80 μg/mL) at room temperature in a 90% humidity atmosphere under 3% CO_2 for two days. Prior to fluorescence imaging experiments, the cells were washed with a PBS buffer (20 mmol, pH7.4) for three times and then incubated again with the NRQDs-Cu^{2+} probes (100 μg/mL) at room temperature for 45 min. Subsequently, the samples were washed with a PBS buffer (20 mmol, pH7.4) three times to eliminate the remaining probes. For the negative and positive control samples, before incubation with the nanoprobes, the A549 cells were initially pretreated with a1.0 mmol/L N-methylmaleimide solution (NMM, which is a thiol-reactive reagent for reducing the Cys level) or Cys (0.5 mmol/L) at room temperature for 2 h. After rinsing with a PBS buffer solution three times, the resulting A549 cells were further incubated with the probes at room temperature for 45 min. After