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Research Paper

A reversible water-soluble naphthalimide-based chemosensor for imaging of cellular copper(II) ion and cysteine

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ABSTRACT

Cellular free copper ion is essential yet potentially toxic to living orgasms, thus cells have evolved mechanisms to balance global copper content including the chelation reactions by cysteine. To better understand the delicate counter-balance of free copper(II) ion and cysteine in the living cells, we developed a water-soluble naphthalimide-based derivative **NC** as a chemosensor for reversible dual detection of copper(II) ion and cysteine. The chemosensor **NC** exhibits a rapid, sensitive and quantitative response for copper(II) ion in aqueous solution due to the formation of the corresponding complex **NC**-Cu(II) in the range of 0–5 μM with the detection limit of 7.11 nM. Afterwards, **NC** is released from **NC**-Cu(II) complex accompanied with a "turn-on" fluorescence in the presence of cysteine ranging from 0 to 160 μM. It's also worth noting that the linear range of the **NC**-Cu(II) complex for the cysteine is identical with the intracellular level of cysteine under physiological conditions (30–200 μM). To achieve its practical application, we further demonstrated that the chemosensor **NC** exhibits good cell permeability and could be employed to monitor free copper(II) ion and cysteine in the living cells.

1. Introduction

Copper is an indispensable trace transition element to human health. It has been involved in many physiological processes, such as the production of hemoglobin, synthesis of elastic protein, metabolism of connective tissue and the formation of nervous tissue [1–4]. The deficiency and excessive intake of copper will cause a variety of diseases, such as: anemia, arthritis, and Parkinson's disease [5–12]. Thus cells have evolved mechanisms to balance global copper content. When copper is transported into cells, several copper involved pathways including cysteine (Cys) coordination reaction are responsible for chelating, thus balancing the release of toxic free copper ion and distributing copper to different subcellular compartments [13–16]. On the other hand, cysteine (Cys), one of the thiol-containing amino acids plays a pivotal role in many biological processes such as reversible redox reaction and cellular detoxification [17–21]. The intracellular level of cysteine (Cys) has been linked to lots of diseases, such as Alzheimer's disease, AIDS and Cancers [22–27]. Thus, effective analytical approaches aimed at simultaneous monitoring cellular free copper(II) ion and cysteine (Cys) are desired to elucidate their behavior in healthy and disease status.

Among various analytical methods to detect cellular copper(II) ion or cysteine (Cys), fluorescence methods have attracted great attention due to their high sensitivity, non-invasiveness and high spatial and temporal resolution [28–31]. As copper(II) ion is a paramagnetic ion with an unfilled d shell and it could quench the fluorescence of the fluorophore to a great extent via electron transfer or energy transfer, numbers of probes have been developed [32–45]. However, some showed low sensitivity [39,40,43]. And most of them functioned well merely in aqueous solution with the assistance of organic solvents such as CH_3CN, CH_3CH_2OH and DMF. [36,37,40,41,44] So, it is necessary to develop a fast response, high sensitive chemosensor for detecting of copper(II) ion in absolute aqueous solution. Besides, it is more challenging to develop a water-soluble indicator to simultaneously image cellular copper(II) ion and cysteine (Cys).

Herein, we designed and synthesized a reversible naphthalimide-based fluorescent chemosensor **NC** for dual detection of the copper(II) ion and cysteine (Cys). The **NC** was composed by naphthalimide and 2-Picolylamine which could act as an efficient "on-off" sensor for copper(II) ion with high sensitivity in neutral aqueous solution, and the **NC**-Cu(II) complex was an "off-on" sensor for cysteine (Cys). The results demonstrated

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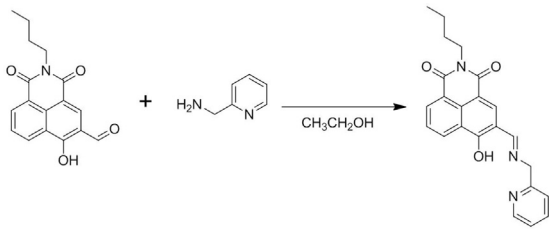

Scheme 1. Synthetic route of **NC**.

that **NC** is very sensitivity for copper(II) ion with a detection limit of 7.11 nM. Meanwhile, the linear range of the **NC**-Cu(II) complex for the cysteine (Cys) is identical with the intracellular level of cysteine (Cys) under physiological conditions (0–160 μM). More importantly, we have demonstrated that the chemosensor **NC** exhibited good cell permeability and could be successfully applied for monitoring copper(II) ion and cysteine (Cys) in the living cells.

2. Experimental

2.1. Materials and instrumentals

The 3-formyl-4-hydroxy-1,8-naphthalimide was prepared according to our previous work [46]. All other chemicals used in this paper were obtained from commercial suppliers and used without further purification. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. NMR spectra were recorded on a Bruker Avance III at 400 MHz for ^{1}H NMR with chemical shifts reported as ppm (in DMSO, TMS as internal standard). Mass spectra (MS) were measured with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10 mm quartz cuvette. pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30). Fluorescence imaging was observed under an Olympus IX81 confocal fluorescence microscope. The absolute fluorescence quantum yield values were measured using Hamamatsu Photonic Multi-Channel Analyzer PMA-12.

2.2. Synthesis of *NC*

3-formyl-4-hydroxy-1,8-naphthalimide (80 mg, 0.27 mmol) and 2-Picolylamine (44 mg, 0.4 mmol) was dissolved in 15 mL absolute ethanol. The mixture was stired at normal temperature for 2 h. After the reaction, the solvent was removed under reduced pressure. The brown residues were purified by silica gel column chromatography using dichloromethane as eluent to afford light-yellow product (Scheme 1). ^{1}H NMR (d6-DMSO,400 MHz): δ($\times10^{-6}$): 13.08 (d, 1H), 8.84 (d, 1H), 8.63 (d, 1H), 8.54 (d, 1H), 8.43-8.34 (m, 2H), 7.88 (d, 1H), 7.63 (t, 1H), 7.50 (d, 1H), 7.40 (d, 1H), 5.04 (d, 2H), 4.09-3.94 (m, 2H), 1.64-1.53 (m, 2H), 1.40-1.26 (m, 2H), 0.99-0.84 (m, 3H). ^{13}C NMR (d6-DMSO, 100 MHz): δ ($\times10^{-6}$): 179.83, 166.86, 164.13, 163.11, 155.35, 149.91, 141.02, 137.78, 132.73, 131.52, 129.10, 125.56, 123.65, 122.75, 122.29, 110.22, 106.64, 55.00, 30.27, 20.31, 14.22. ESI-HRMS calcd for $C_{23}H_{21}N_3O_3$ [M+H]$^+$: 388.1660, found 388.1656.

2.3. General procedure for analysis

Parent stock solution of fluorescent probe **NC** (2.0 mM) was prepared in DMSO. The solution of test were prepared by placing 25 μL of parent stock solution into the test tube, then diluting the solution to 5 mL with aqueous solution containing HEPES (5 mM, pH = 7.0). All spectra were obtained in a quartz cuvette (path length = 1 cm). Cu^{2+} was obtained by the anhydrous cupric sulfate. The solution of Zn^{2+}, Pb^{2+}, Ni^{2+}, Mg^{2+}, Ag^+ were prepared from their nitrate salts. Al^{3+}, Ca^{2+}, Fe^{3+}, Fe^{2+}, Na^+, K^+, Mn^{2+}, Cr^{3+}, Hg^{2+} were prepared from their chloride salts. All the amino acids were purchased from Sigma-Aldrich Chemical.

2.4. Determination of the detection limit

This calculation method referred to the previous paper, the detection limit was calculated based on the fluorescence titration [47]. Fluorescence titration was carried out in the aqueous buffer (5 mM HEPES, pH = 7.0) to determine the detection limit, which was then calculated with the following equation:

$$\text{Detectionlimit} = 3\sigma/k$$

where σ is the standard deviation of blank measurements, k is the slope between the fluorescence intensity *vs* Cu^{2+} or Cys concentration.

2.5. Cell culture and imaging

Hela cells were grown on glass-bottom culture dishes using DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 50 μg mL^{-1} penicillin-streptomycin in a humidified 37 °C, 5% CO_2 incubator. Before use, the adherent cells were washed three times with FBS-free DMEM. The cells were incubated with 5 μM **NC** in culture media for 30 min at 37 °C and then washed with PBS (pH 7.4) twice. Fluorescence imaging of Hela cells was observed under an Olympus IX81 confocal fluorescence microscope, and the excitation wavelength was 405 nm.

3. Results and discussion

The fluorescence and absorption spectra of **NC** towards copper(II) ion was verified in the aqueous buffer (5 mM HEPES, pH = 7.0). As shown in Fig. S1, after the addition of copper(II) ion (30 μM), the absorption peak of **NC** at 400 nm was decreased, revealing the coordination of **NC** and copper(II) ion. Meanwhile, the fluorescence band centered at 520 nm was decreased sharply (excited by 400 nm), due to the heavy metal effect [48–51]. As shown in Fig. S2, after the addition of Cys (200 μM) to the solution of **NC**-Cu(II) complex (10 μM Cu^{2+}–10 μM of **NC** solution in the aqueous buffer (5 mM HEPES, pH = 7.0)), the recovery at 400 nm was observed. Meanwhile, the fluorescence band centered at 520 nm was increased sharply. This results indicated the release of **NC** from the **NC**-Cu(II) complex through the chelation of Cys with the copper(II) ion. Next, the reversibility of **NC** was further tested. As shown in Fig. 2, the alternate additions of constant concentration of copper(II) ion and Cys to the **NC** solution aroused the switchable fluorescence changes at 520 nm. Such a reversible cycle could be repeated at least for 3 times by the alternate change of copper(II) ion and Cys addition, implying that **NC** could be used as a reversible fluorescence ON-OFF-ON chemosensor to monitoring free copper(II) ion and Cys.

Then, the quantitative analysis assay of copper(II) ion was investigated in detail. As shown in Fig. 1b, continuous addition of copper(II) ion to the solution of **NC** resulted in a gradual decrease of the fluorescence band centered at around 520 nm. The linear relationship between the fluorescence intensities at 520 nm and the copper(II) ion concentrations in the range of 0–5 μM was obtained. Therefore, **NC** possesses excellent sensitivity and can quantitatively detect copper(II) ion in the aqueous solution. The detection limit was determined to be as low as 7.11 nM. The absorption spectra at 400 nm was also gradual decrease with the increase addition of