Sensors and Actuators B 233 (2016) 639–645

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A water-soluble fluorescent probe for ClO^- and Cd^{2+} under physiological pH and its applications in living cells imaging

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 21 April 2016
Accepted 25 April 2016
Available online 27 April 2016

Keywords:
Fluorescence probe
Fluorescein
Water-soluble
ClO^-
Cd^{2+}
Living cells

ABSTRACT

A water-soluble fluorescence probe (TF) derived from fluorescein has been developed. This probe can be used to detect ClO^- and Cd^{2+} under physiological condition based on oxidation and coordination reactions, exhibiting almost 64-fold and 4-fold fluorescence enhancement. The experimentally observed changes in the structure and electronic properties of the probe in the presence of ClO^- and Cd^{2+} were modeled by density functional theory (DFT) and time-dependent density functional theory (TDDFT) computational calculations. Moreover, the present probe was successfully applied to the fluorescence imaging of ClO^- and Cd^{2+} in living cells.

1. Introduction

The development of simple analytical methods for the sensitive detection of reactive oxygen species (ROS) and toxic metals has attracted significant attention due to their effects on health of humans and animals [1–3]. Hypochlorite (ClO^-), as a prominent member of ROS, is widely used, for example, NaClO is frequently used as a disinfectant and bleaching agent. ClO^- plays a critical role in immune system against inflammation and microorganisms [4,5]. However, an excessive amount of ClO^- could be harmful to the health of human beings, and can result in diseases such as arthritis, renal disease, lung injury, atherosclerosis, and cancers [6–10]. Among the toxic metal ions, cadmium ion (Cd^{2+}) is an occupationally and environmentally toxic ion which is widely used in metallurgy, military industry and agriculture [11,12]. Cd^{2+} has a long elimination half-life (10–30 years) in the human body and is classified as a potent carcinogen [13]. Cd^{2+} can cause serious injury to the human lungs, kidneys, bone and nerve system, which results in bone disease, heart disease and diabetes [14,15]. In addition, ROS are also implicated in cadmium toxicology [16]. Therefore, a rapid and sensitive method for monitoring ClO^- and Cd^{2+} in biological systems is highly important.

Fluorescent probes have been evaluated as powerful tools to detect chemical agents in recent years, due to their high sensitivity, high selectivity, easy operation and fast response, and were used not only in conventional experiments but also in vivo studies [17–23]. Especially for the living biological imaging research, the probes are advantageous because they cause less cell damage while displaying high spatial and time resolution capabilities in visualizing analytes [24,25]. Hence, the design and synthesis of novel fluorescent probes, which can recognize these anions and cations, are desirable and significant. Up to now, a number of fluorescent probes for ClO^- [26–32] and Cd^{2+} [33–35] have been reported. However, those probes might encounter some problems, such as poor water solubility, weak selectivity, low quantum yield and a relatively short emission wavelength. Therefore, there is still need to further develop water-soluble, selective, and sensitive fluorescent probes for ClO^- and Cd^{2+}.

Fluorescein (C.I. Solvent Yellow 94) was widespread used in many areas, for example, as a fluorescent probe in biomarkers and detecting eye diseases [36,37]. It has high fluorescence quantum yield, high solubility in water, visible excitation and emission wavelength, low toxicity and strong interaction with biomolecules. Thus, in this work, we report a novel and water-soluble fluorescein-based

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http://dx.doi.org/10.1016/j.snb.2016.04.142
0925-4005/

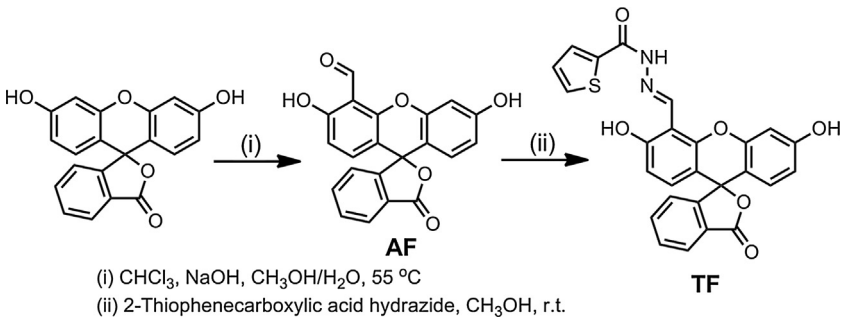

Fig. 1. Synthetic route of probe TF.

fluorescent probe for detection of ClO^- and Cd^{2+} (Fig. 1). This probe (TF) was derived from Schiff base fluorescein and exhibited highly selective and sensitive response to ClO^- and Cd^{2+} in aqueous solution. The fluorescent changes are mainly due to the redox reaction of TF and ClO^- and coordination reaction of TF and Cd^{2+}, which were reasonably proved by combined experimental and computational study. Furthermore, the probe can also used to living cells imaging.

2. Experimental details

2.1. Chemicals and reagents

Fluorescein (95%) and 2-thiophenecarboxylic acid hydrazide (97%) were purchased from J&K Chemical Ltd. NaClO (5% available chlorine), KI, NaCl, KBr, NaF, CH_3COOH, NaN_3, NaN_3, $NaNO_2$, Na_2S, H_2O_2, $CdSO_4{\cdot}8/3H_2O$, LiCl, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, $MnSO_4$, $FeCl_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $Hg(NO_3)_2$, $CuCl_2{\cdot}5H_2O$, and $AlCl_3{\cdot}6H_2O$ were purchased from Sinopharm Chemical Reagent Co., Ltd. Other reagents were of analytical reagent grade and used without further purification or treatment. All aqueous solutions were prepared with ultrapure water obtained by a Milli-Q water purification system (18.2 MΩ cm). A stock solution of 1 mmol L^{-1} ClO^- was quantified by iodometry. ClO^- solutions at various concentrations were prepared by diluting the stock solution with ultrapure water. The living HeLa (human cervical adenocarcinoma) cells were provided by the Jilin University hospital of stomatology (China).

2.2. Instruments

UV–vis and fluorescence spectra were recorded on a TU-1810C UV–vis spectrometer (Beijing Purkinje General Inc., China) and a VARIAN Cary Eclipse spectrofluorophotometer (Agilent Technologies, USA), respectively. Spectra of ^1H NMR (TMS as internal standard) were measured on a Mercury 300BB nuclear magnetic resonance spectrometer (Varian Inc., USA). Mass spectra were measured on a LC/MS QTRAP spectrometer (AB SCIEX Inc., USA). FT-IR spectrum was recorded using KBr pellets on a Nicolet Avatar 360 FT-IR spectrophotometer (Thermo Fisher Scientific Inc., USA) in the wavenumber region of 4000–400 cm^{-1}. All pH measurements were made with a PHS-3C pH-Meter (INESA Scientific Inc., China). Cells were imaged by an Olympus IX 51 inverted fluorescence microscope (Olympus Corporation, Japan) equipped with integrated color filters.

2.3. Synthesis of probe TF

The synthetic route of TF is shown in Fig. 1.

2.3.1. Synthesis of AF

AF was synthesized from fluorescein according to the reported method [38]. 2.0 g of fluorescein (6 mmol) and 3.5 mL of CH_3OH were put into a 100 mL bottom flask. Then 8.5 mL of 50% NaOH solution, 2.0 mL of $CHCl_3$ (25 mmol), and 30 mg of dibenzo-18-crown-6 (catalytic amount) were added in the flask. The reaction temperature was maintained at 55 °C and the mixture was stirred for 16 h. Having been cooled, the mixture was acidified with 8 mol L^{-1} H_2SO_4. The precipitate was purified by chromatography on a silica gel column with CH_2Cl_2-ethyl acetate (10:1, *v:v*) as the elution solvent. Finally, 0.67 g of AF as a light yellow solid (yield 31%) was obtained. ESI-HR-MS, *m/z*: 383.1468 $[M+Na]^+$ (calcd. 383.0526 for $C_{21}H_{12}NaO_6^+$). ^1H NMR (300 MHz, DMSO-*d6*), δ: 11.88(s, 1H), 10.63(s, 1H), 10.26(s, 1H), 8.02(d, 1H), 7.85-7.70(m, 2H), 7.30(s, 1H), 6.96(d, 1H), 6.85(s,1H), 6.71(d, 1H), 6.61(s, 2H).

2.3.2. Synthesis of TF

0.18 g of AF (0.5 mmol) and 71 mg of 2-thiophenecarboxylic acid hydrazide (0.5 mmol) were mixed in 10 mL of CH_3OH, following by stirring at room temperature for 12 h. The resulting precipitate was filtered and washed 3 times with 15 mL CH_3OH. Finally, 0.18 g of TF as dark yellow solid (yield 75%) was obtained. ESI–MS, *m/z*: 485.0 $[M+H]^+$. FT-IR (KBr, cm^{-1}): 3453, 1753, 1633, 1354, 1282, 1221, 1165, 1119. ^1H NMR (300 MHz, DMSO-*d6*), δ: 12.63 (s, 1H), 12.54 (s, 1H), 10.31 (s, 1H), 9.20 (s, 1H), 8.11-7.90 (m, 3H), 7.78 (dtd, 2H), 7.38-7.24 (m, 2H), 6.80-6.56 (m, 5H).

2.4. Spectroscopic experiments

The probe TF was dissolved in DMF to get a 10 mmol L^{-1} stock solution. Before spectroscopic measurement, the solution was freshly prepared in HEPES aqueous buffer (50 mmol L^{-1}, pH = 7.4) by diluting the high concentration stock solution to 10 μmol L^{-1}. ClO^- and Cd^{2+} were detected by the addition of different concentration stock solutions of ClO^- and Cd^{2+} to TF (10 μmol L^{-1}) solution. The fluorescence emission spectra were recorded in the wavelength range of 500–650 nm with excitation wavelength at 495 nm. Both the excitation and emission slits were set at 2.5 nm/2.5 nm for ClO^- and 2.5 nm/5.0 nm for Cd^{2+}, respectively.

2.5. Preparation of cells

The HeLa Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, incubated under an humidified atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. Cells were seeded on dish for fluorescence microscopic imaging by inversion fluorescence microscope.