Materials Science and Engineering C 72 (2017) 551–557

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

A novel fluorescence probe based on triphenylamine Schiff base for bioimaging and responding to pH and Fe^{3+}

Lei Wang [a], Xiaodong Yang [a], Xiuli Chen [a], Yuping Zhou [b], Xiaodan Lu [c], Chenggong Yan [c], Yikai Xu [c], Ruiyuan Liu [b,*], Jinqing Qu [a,*]

[a] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, PR China
[b] Guangdong Provincial key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, PR China
[c] Medical Imaging Center, Nanfang Hospital, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 25 October 2016
Accepted 23 November 2016
Available online 5 December 2016

Keywords:
Fluorescence probe
Triphenylamine
Bioimaging
Schiff base

ABSTRACT

A novel fluorescence probe **1** based on triphenylamine was synthesized and characterized by NMR, IR, high resolution mass spectrometry and elemental analysis. Its fluorescence was quenched when pH below 2. There was a linear relationship between the fluorescence intensity and pH value ranged from 2 to 7. And its fluorescence emission was reversibility in acidic and alkaline solution. Furthermore, it exhibited remarkable selectivity and high sensitivity to Fe^{3+} and was able to detect Fe^{3+} in aqueous solution with low detection limit of 0.511 μM. Job plot showed that the binding stoichiometry of **1** with Fe^{3+} was 1:1. Further observations of ^1H NMR titration suggested that coordination interaction between Fe^{3+} and nitrogen atom on C═N bond promoted the intramolecular charge transfer (ICT) or energy transfer process causing fluorescence quenching. Additionally, **1** was also able to be applied for detecting Fe^{3+} in living cell and bioimaging.

1. Introduction

The pH value in different cellular compartments, body fluids and organs is usually tightly regulated in a process called acid–base homeostasis, which play an irreplaceable role in maintaining normal morphology and function of cell and organism and keeping health of human beings [1]. Under normal physiological conditions, the pH reflecting the concentration of hydronium ion is around 1.0 for gastric acid, 4.5 for lysosomes, 5.5 for human skin and granules of chromaffin cells, 6.0 for urine, 7.2 for cytosol, 7.5 for cerebrospinal fluid and mitochondrial matrix, and 8.1 for pancreatic secretions [2]. Intracellular pH plays an extremely vital role in biological processes occurring in live cells and tissues such as proliferation and apoptosis [3–5], enzymatic activity [6,7], ion transport [8,9], muscle contraction [10,11], endocytosis and multidrug resistance [1,12]. Abnormal pH variation signifies intimate connection multiple cellular immune dysfunction and will cause a lot of human physiology and pathophysiology diseases such as cancer, Alzheimer's disease and cardiopulmonary problems [13–15]. Hence, the monitoring of pH change is a significant goal in the field of observing cell metabolic activity, diagnosing physiological disease and investigating pathological process.

Transition metal ions play a critical role in human life. Among many biologically significant metal ions, Fe^{3+} is one of the most abundant and essential metal ions in the human body because it is involved in many biochemical processes, provides the oxygen carrying capacity to heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain [16–18], as well as participates in electron transfer [19,20] and oxidation reactions [21,22]. Nevertheless, both its deficiency and excess can induce a variety of diseases in the human body, such as anemia [23], cancer [24–26], dysfunction of liver [27,28] and kidney [29,30], Parkinson's disease [31,32], Alzheimer's disease [33–35] and hemochromatosis [36]. Therefore, various efficient chemosensors specific for Fe^{3+} detection have attracted the attention of scientists.

Over the past decades, considerable pH-sensitive probes based on fluorescence responses have been developed according to different operating principles. First of all, alkyl and aromatic amines are typically substances for pH sensors due to the highly sensitive towards acid and the exceptionally rapid rates of proton transfer occurring in acid–base equilibrium processes [1]. Secondly, *N*-heterocyclic derivatives, such as pyridine, quinolone, indole, pyrrole, imidazole, are the key component of plentiful pH probes. Another group involved in pH probes is phenol hydroxyl [37–40]. The main mechanism of these fluorescence probes for detection pH is protonation or deprotonated process in acid or basic environments. Additionally, some other fluorescence pH chemosensors have been designed on the basis of chemical reactions such as additions, isomerizations or ring opening processes [1,12].

However, a majority of pH fluorescent probes respond to neutral pH ranging from 6 to 8 [41–43] and weakly acid pH ranging from 4 to

* Corresponding authors.
E-mail addresses: ruiyliu@smu.edu.cn (R. Liu), cejqqu@scut.edu.cn (J. Qu).

http://dx.doi.org/10.1016/j.msec.2016.11.108
0928-4931/

6 [44–46]. Very few probes are sensitive to pH value below 4 [47,48] and suitable for extremely acid conditions [49]. Therefore, the detection of the strong acid (pH value below 2) conditions is still a difficult problem to be solved. Another issue is that most sensors have no selectivity in recognition of different cells except quite a few fluorescent biosensor for targeted imaging of cancer cells [50] and specific mitochondrial imaging to identify differentiating brown adipose cells [48]. In addition, the pH probe for simultaneously detecting Fe^{3+} is scarce [47].

Herein, a novel fluorescence probe based on triphenylamine was synthesized via nucleophilic addition reaction between 4-(diphenylamino)benzaldehyde and 1,4-diaminobutane, which could be dispersed well in aqueous solution and was sensitive to the variation of pH value. Especially, its fluorescence was quenched when pH value below 2 and it exhibited a linear relationship between the fluorescence intensity and pH from 2 to 7. Furthermore, it displayed remarkably selective and highly sensitive towards Fe^{3+} in aqueous solution. Compared with numerous chemosensors developed through sophisticated synthetic approaches or containing heavy metal pollutant, the current probe **1** was synthesized easily without heavy metal in one step and the synthetic pathway is depicted in Scheme 1.

2. Experimental section

2.1. Materials

All chemicals and solvents were commercially available and used directly without further purification unless specified. All kinds of organic solvent (including absolute methanol, dimethylsulfoxide, dichloromethane) were purchased from Guangdong Guanghua Sci-Tech Co., Ltd. 1,4-Diaminobutane (Aladdin, 99%), 4-(diphenylamino)benzaldehyde (Adamas, 98%).

The solutions of metal ions were prepared from KNO_3, $Ca(NO_3)_2$, $NaNO_3$, $Mg(NO_3)_2$, $Fe(NO_3)_3{\cdot}9H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Pb(NO_3)_2$, $Hg(NO_3)_2$, $AgNO_3$, $Fe(NO_3)_2$, $Co(NO_3)_2$, $Mn(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2{\cdot}9H_2O$ and $Ni(NO_3)_2{\cdot}6H_2O$, respectively. **1** was dissolved in DMSO at room temperature to afford the stock solution (100 μM). Fluorescence spectra were obtained using a Hitachi F-4500 spectrofluorometer with a xenon lamp and 1.0 cm quartz. The fluorescence spectra were obtained by excitation at 365 nm. The excitation and emission slit widths were 2.5 nm and 5 nm, respectively.

2.2. Instrumentation

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer with $CDCl_3$ as solvent and tetramethylsilane as an internal reference. IR spectra were measured using a Shimadzu FTIR-8100 spectrophotometer. Melting points (Mp) were measured on a Yanaco micro-melting point apparatus. High resolution mass spectra (HR-MS) were carried out on a Bruker spectrometer using ESI ionization. Elemental analysis was performed on an Eager 300 elemental microanalyzer. UV–vis spectra were recorded in a quartz cell (thickness: 1 cm) at room temperature using a Shimadzu UV-2450 spectropolarimeter. Fluorescence spectra were obtained using a Hitachi F-4500 spectrofluorometer with a xenon lamp and 1.0 cm quartz.

2.3. Synthesis of probe 1

4-(Diphenylamino)benzaldehyde (2.7348 g, 10 mmol) and 1,4-diaminobutane (0.4426 g, 5 mmol) were dissolved in absolute methanol (60 mL) and refluxed for 12 h at 65 °C (Scheme 1). Then, the white precipitate was filtrated, washed three times with absolute methanol and dried in vacuum to give **1** (2.4223 g, 81%).

^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.18 (s, 1H), 7.56 (d, $J = 8.1$ Hz, 2H), 7.27 (t, $J = 7.0$ Hz, 4H), 7.11 (d, $J = 7.8$ Hz, 4H), 7.05 (t, $J = 9.3$ Hz, 4H), 3.62 (s, 2H), 1.74 (d, $J = 9.1$ Hz, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.4, 150.0, 147.2, 129.9, 129.4, 129.1, 125.1, 123.6, 122.1, 61.5, 28.8.

IR (v^{-1}, KBr): 3670, 3059, 3027, 2938, 2829, 1642, 1588, 1485, 1328, 1273, 1173, 831, 756, 693, 520.

HR-MS (ESI): $C_{42}H_{38}N_4$ m/z, 598.3096 for [M + H]+: 599.3169.

Elemental analysis: Calcd C, 84.25; H, 6.40; N, 9.36. Found C, 84.39; H, 6.48; N, 9.13.

Mp: 185.3–185.9 °C.

2.4. Absorption and fluorescence analysis

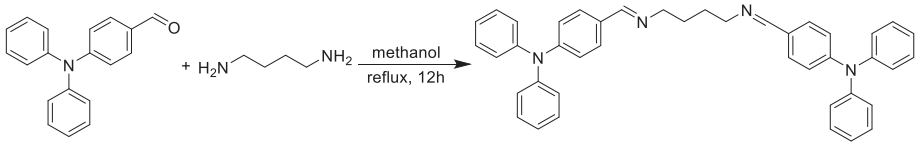
A typical experimental procedure was described as follows: Stock solutions of probe **1** (DMSO, 100 μM) was prepared in a 10 mL volumetric flask. 100 μL stock solution of probe **1** (100 μM) was transferred to a 10 mL volumetric flask and diluted to volume with twice-distilled water to give the sample solution. The concentration of probe **1** in the sample solution was 1 μM throughout the analysis experiments unless otherwise stated. All the test solutions were measured at room temperature after 30 min of preparation.

2.5. MTT assay

Cytotoxicity studies were performed using MTT assay. HeLa cells (10^6 cells/mL) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2/95% air incubator for 4 h. **1** was diluted to different concentrations of solution with medium and added to each well after the original medium has been removed. HeLa cells were incubated with probe concentrations for 24 or 48 h. The concentrations of the probe were 1.25 to 40 μM. 200 μL MTT solution (5.0 mg/mL, HEPES) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a TRITURUS microplate reader.

2.6. Cellular imaging

HeLa cells were grown in RPMI 1640 medium supplemented with 10% FBS (Fetal Bovine Serum) at 37 °C in humidified environment of 5% CO_2. Cells were plated on 6-well plate at 5×10^4 cells per well and allowed to adhere for 12 h. **1** dissolved in DMSO (0.5 μL, 5 mM) were added to the cells medium (500 μL) at 5 μM final concentrations. After incubating for 30 min, excess **1** was removed by gentle rinsing with



Scheme 1. Synthesis of probe **1**.