Journal of Photochemistry and Photobiology A: Chemistry 335 (2017) 1–9

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Novel reversible fluorescent probe for relay recognition of Zn^{2+} and PPi in aqueous medium and living cells

Zhen Ming Dong, Wei Wang, Li Yuan Qin, Jia Feng, Jia Na Wang, Yu Wang*

School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, PR China

ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form 22 October 2016
Accepted 3 November 2016
Available online 15 November 2016

Keywords:
Fluorescent chemosensor
Recognition
Zn^{2+}
PPi
Cell imaging
Aqueous medium

ABSTRACT

A new fluorescent probe 4′-hydroxy-3′-((8-quinolineimino) methyl)-4-biphenylcarbonitrile (L) has been synthesized for the sequential detection of Zn^{2+} and PPi in aqueous ethanolic (3/2 v/v) HEPES buffer (pH 7.1). Addition of Zn^{2+} to the solution of L resulted in a red shift ($\Delta\lambda = 18$ nm) with a pronounced enhancement in the fluorescence intensity, while there was no enhancement in presence of other metal ions. The subsequent addition of PPi anion can switch off the fluorescence signal by bringing the Zn^{2+} ion out of the coordination cavity of the chemosensor L. No interference was observed from other anions, making the L–Zn^{2+} ensemble a highly sensitive and selective PPi probe. The detection limit is 5.31×10^{-8} M and 9.41×10^{-8} M for Zn^{2+} and PPi, respectively. The sensing process is reversible and the probe may be recycled for further use. Cell imaging studies demonstrate that this sensor is capable of sensing Zn^{2+} and PPi in living cells.

1. Introduction

The recognition and sensing of biologically and chemically important ions (cations, anions) is an important area of chemical research. Fluorescence-based sensing method has been preferred over other methods due to its high sensitivity, short response time and most importantly capability of real time detection. Zinc is the second most abundant transition metal ion in the human body after iron and plays crucial roles in many biological activities including gene expression, cellular metabolism, neurotransmission and apoptosis. The disorder of zinc metabolism is associated with many severe diseases such as epilepsy [1], hypoxia ischemia [2] and Alzheimer's diseases [3]. Therefore, sensitive real-time detection and imaging of zinc ion in biological systems is of significant interest and importance. Although numerous fluorescent chemosensors for Zn^{2+} ion have been designed and developed to date [4–23], limitations exist such as complicated multistep synthesis process, insufficient selectivity and/or sensitivity. Thus, development of easily prepared and water-soluble zinc sensor of improved sensitivity and selectivity continues to be a growing area of interest [24,25].

On the other hand, as the product of ATP hydrolysis under cellular conditions, pyrophosphate (PPi) is involved in several biological processes including cellular signal transduction, gene transcription and protein synthesis [26]. PPi concentration can provide critical information on important processes such as DNA replication and hence can be used in cancer diagnosis by monitoring telomerase elongation process [27]. Also, high synovial fluid PPi is associated with some diseases such as formation of calcium pyrophosphate dehydrate crystals and chondrocalcinosis [28]. Therefore, the selective detection of PPi has been a major research focus. Due to high solvation energy of PPi in water ($\Delta G^0 = 584$ KJ mol^{-1}) [29] and the presence of other competitive anions, it is still a challenging task for the chemist to design water soluble fluorescent chemosensors for selective detection of PPi in aqueous medium. In recent times, metal ion complexes have been exploited to develop fluorescence-based phosphate sensors owing to the specific metal ion–anion interactions [30,31]. Among them, Zn^{2+} ensembles for the selective sensing of PPi have attracted considerable attention owing to the strong affinity of Zn^{2+} toward PPi [28,32–40]. However, examples on specific recognition as well as quantitative detection of PPi over Pi and other phosphate anions in aqueous medium and in living cells are still limited in number. Therefore, there is a great demand for the development of efficient PPi optical chemosensors that can work in aqueous media and shows rapid response for effective in vivo detection.

* Corresponding author.
E-mail address: wangyu1168@sxu.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.jphotochem.2016.11.014
1010-6030/

Herein we report a novel 4-biphenylcarbonitrile based fluorescent probe, L on a Schiff base scaffold of 8-aminoquinoline, which was found to display selective fluorescence enhancement toward Zn^{2+} ions in neutral aqueous media. The L–Zn complex was further utilized for PPi sensing by metal displacement approach, ensuing quenching of fluorescence by the reversible return of L from L–Zn complex. In addition to solution based experiments, the detection of Zn^{2+} and PPi ions in HepG2 cells was investigated.

2. Experimental

2.1. Materials and instrumentation

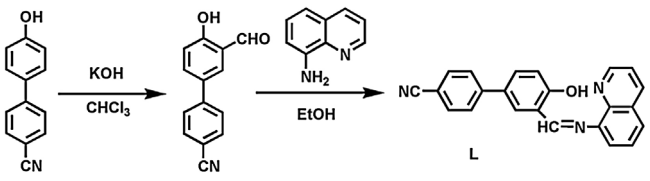
4′-Hydroxy-4-biphenylcarbonitrile (95%) and 8-aminoquinoline (98%) were purchased from Acros Organics Co. (Beijing, China). All the other chemical reagents were of analytical grade and used as received without further purification. The metallic ions were supplied from their corresponding nitrates and the anions from their sodium or potassium salts. Double distilled water was used throughout.

The UV absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed on a LS-55 spectrofluorimeter (PerkinElmer, USA). The samples were excited at 444 nm. IR spectra were taken as KBr pellets on a TENSOR II infrared spectrometer (Bruker, Germany). ^{1}H NMR spectra were recorded on a DRX-600 spectrometer (Fällenden, Switzerland). High resolution mass spectra were obtained on a Bruker micrOTOF-Q III mass spectrometer. The pH measurements were carried out on a pHS-3C acidometer (Shanghai Precision & Scientific Instrument Co., Ltd., China).

2.2. Synthesis of L

L was prepared in two steps from 4′-Hydroxy-4-biphenylcarbonitrile as shown in Scheme 1. 3′-Formyl-4′-Hydroxy-4-biphenylcarbonitrile was initially prepared using the literature method [41]. A white solid was obtained, mp 132–134 °C. ^{1}H NMR (DMSO-*d*6) δ(ppm): 11.07 (s, 1H, OH), 10.34(s, 1H, CHO), 8.03 (s, 1H, ArH), 7.96 (d, 1H, ArH), 7.91 (d, 2H, ArH), 7.87 (d, 2H, ArH), 7.15 (d, 1H, ArH).

In the second step, 3′-formyl-4′-Hydroxy-4-biphenylcarbonitrile (0.11 g, 0.5 mmol) and 8-aminoquinoline (0.072 g, 0.5 mmol) was dissolved in 5 mL of ethanol. The mixture was heated under reflux for 2 h and then cooled to room temperature. The precipitated solid was filtered off and recrystallized from ethanol to give the product as an orange-red solid (0.13 g, 71% yield). IR ν_{max} (KBr) 3417 (O—H), 2228(C≡N), 1624 (C=N), 1605, 1484, 1443 (C=C), 1280, 1191, 1087, 1061 (C—O). ^{1}H NMR (DMSO-*d*6) δ: (DMSO-*d*6) δ: 14.34 (s, 1H, OH), 9.24(s, 1H, CH=N), 9.00 (s, 1H, ArH), 8.47 (d, 1H, ArH), 8.17 (s, 1H, ArH), 7.95 (d, 1H, ArH), 7.94 (d, 2H, ArH), 7.93(d, 2H, ArH), 7.90(m, 1H, ArH), 7.80 (d, 1H, ArH), 7.73(t,1H, ArH), 7.66(m,1H, ArH), 7.14(d,1H, ArH). 13C NMR (DMSO-d6): 164.39, 162.94, 151.19, 144.56, 144.06, 142.14, 136.77, 133.22, 132.57, 131.77, 129.23, 129.01, 127.35, 127.33, 127.14, 122.72, 120.08, 119.45, 118.65, 118.60, 109.73. HRMS(ESI): calcd. for $C_{23}H_{16}N_3O$ (L+H) 350.1293, found 350.1297.



Scheme 1. Synthesis of L.

2.3. UV–visible and fluorescence spectral measurements

Stock solution of L was prepared in ethanol at 1.0×10^{-3} M. All ion stock solutions were prepared at 1.0×10^{-2} M by dissolving appropriate amounts of metal salts in water. These stock solutions were used for different spectroscopic experiments after appropriate dilution. All experiments were carried out in an EtOH—H_2O solution (3:2 v/v, 10 mM HEPES buffer, pH = 7.1). The titrations were performed by successive incremental addition of ion solutions to a fixed volume of receptor L solution in a 10 ml volumetric flask. All fluorescence and absorbance spectra were recorded following 5 min of the addition of the ions. The fluorescence quantum yields of L and its Zn2+ complex were measured by the steady-state comparative method using quinine sulfate as the standard (Φ = 0.54) [23].

2.4. Cell imaging

In vitro experiments were performed using Human Heptocarcinoma Cell Line HepG2. HepG2 cells were cultured in DMEM medium, which was supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 at 37 °C. Immediately before the experiments, the cells were incubated with 20 μM L in 0.1 M sterile HEPES buffer for 30 min at room temperature. After incubation, the cells were washed with HEPES buffer and incubated with Zn^{2+} (40 μM) for additional 30 min at room temperature. Finally, the cells treated with L and Zn^{2+} were further incubated with KPPi (100 μM) for another 30 min, and then washed with HEPES buffer (2 mL × 3) before observation. The fluorescence images were captured on an Olympus FV1000 confocal microscope (Tokyo, Japan) equipped with a PMT1 detector and with a filter that allowed yellow light emission. The cells were examined and photographed using an objective lens of 40 × magnification (excited at 405 nm).

3. Results and discussion

3.1. Spectroscopic studies of L toward Zn^{2+}

Bearing two nitrogen atoms (imine N and quinoline N) and a phenol —OH group in the vicinity, It was anticipated that L may exhibit selective response to Zn^{2+} along with Zn^{2+} binding induced enhancement of the rigidity of the molecular assembly. To get an insight into the binding property of L toward Zn^{2+}, the spectroscopic properties of L were investigated in an ethanol-water (3:2, v/v) (HEPES buffer, pH = 7.1). As depicted in the spectrophotometric titration curve (Fig. 1a), free L exhibited an intense band at 286 nm ($\varepsilon = 2.73 \times 10^4\ M^{-1}\ cm^{-1}$) and a weak band at ca. 327 nm ($\varepsilon = 7.39 \times 10^3\ M^{-1}\ cm^{-1}$), possibly originating from intraligand transitions and the long conjugation present in the free ligand system. On incremental addition of Zn^{2+} ions (0–80 μM) to the solution of the probe the absorption band at 286 nm decreased whereas the peak at 327 nm increased gradually. In addition, a new absorption peak at ca. 438 nm ($\varepsilon = 6.09 \times 10^3\ M^{-1}\ cm^{-1}$) gradually developed and the color of the solution turned from colorless to yellow (Inset, Fig. 1a). The spectral observation undoubtedly suggests the conversion of free L to the corresponding Zn^{2+} complex. The presence of isosbestic point (298 nm) indicates that the formation of a new species having a certain stoichiometry between L and Zn^{2+}formed in solution. The new band at 438 nm could be attributed to the removal of phenolic proton in the L-Zn complex [11].

In the case of emission studies, L showed the maximum intensity at 524 nm at 327 nm excitation wavelength with a low