Talanta 79 (2009) 327–332

Contents lists available at ScienceDirect

Talanta

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

Salicylaldehyde hydrazones as fluorescent probes for zinc ion in aqueous solution of physiological pH

Na Li, Yu Xiang, Xiaotong Chen, Aijun Tong*

The Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Department of Chemistry, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 12 November 2008
Received in revised form 20 March 2009
Accepted 25 March 2009
Available online 5 April 2009

Keywords:
Ratiometry
Fluorescent probe
Zinc ion
Salicylaldehyde hydrazone

ABSTRACT

Salicylaldehyde hydrazones of **1** and **2** were synthesized and their potential as fluorescent probes for zinc ion was investigated in this paper. Both of the probes were found to show fluorescence change upon binding with Zn^{2+} in aqueous solutions, with good selectivity to Zn^{2+} over other metal ions such as alkali/alkali earth metal ions and heavy metal ions of Pb^{2+}, Cd^{2+} and Hg^{2+}. They showed 1:2 metal-to-ligand ratio when their Zn^{2+} complex was formed. By introducing pyrene as fluorophore, **2** showed interesting ratiometric response to Zn^{2+}. Under optimal condition, **2** exhibited a linear range of 0–5.0 μM and detection limit of 0.08 μM Zn^{2+} in aqueous buffer, respectively. The detection of Zn^{2+} in drinking water samples using **2** as fluorescent probe was successful.

1. Introduction

Zinc ion, which serves as an essential ingredient for various enzymes, is a very important ion species in many biological activities [1,2]. It is the second most abundant heavy metal ion after iron in human body with its total content of 2–3 g in whole body and concentration as high as 10 μM in serum [3]. Because of these, as well as the simple instrumentation and visible signal advantages of fluorescence technology, the quantification of Zn^{2+} by artificial fluorescent probes in environmental or biological samples has attracted much attention in recent years [4–9].

There have been many excellent Zn^{2+}-selective fluorescent probes reported during the latest two decades. However, most of them give fluorescence enhancement response upon the interaction with Zn^{2+} [10–18], with only a few of them that could sense Zn^{2+} ratiometrically [19–22]. It is generally believed that fluorescent probes of ratiometric type exhibit advantages of better resistance to variation of sensor concentration, higher sensitivity, and signal of color change [23]. Thus, the design and development of these probes are of great concern and challenge.

In this paper, we investigated the potential of some salicylaldehyde hydrazones (**1** and **2**) as fluorescent probes for Zn^{2+}, because it was reported that salicylaldimines underwent fluorescence enhancement upon chelating with Zn^{2+} [24–26]. The salicylaldehyde hydrazone moiety [27,28] served as both receptor and reporter in Zn^{2+} sensing, with its advantage of high selectivity toward Zn^{2+} over other metal ions. These salicylaldehyde hydrazones showed 1:2 metal-to-ligand ratio, respectively, when their Zn^{2+} complex formed. Moreover, by introducing pyrene as donor, ratiometric fluorescent probe (**2**) for Zn^{2+} was also constructed. The application of probe **2** for determination of Zn^{2+} in water samples by fluorescence ratiometry was also successful.

2. Experimental

2.1. Reagents

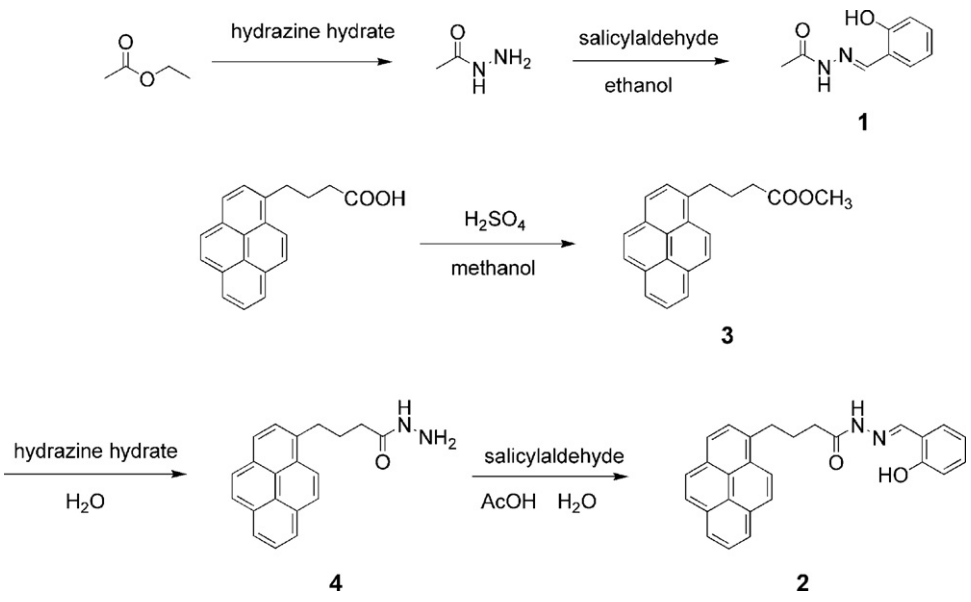
Absolute ethanol of analytical grade and deionized water (distilled) were used throughout the experiment as solvents. All the materials for synthesis were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from their nitrate salts of analytical grade, and then subsequently diluted to prepare working solutions. Tris–HCl buffer solutions of different pH were prepared using proper amount of Tris and HCl (all of analytical grade) under adjustment by a pH meter.

2.2. Apparatus

Absorption spectra were determined on a JASCO V-550 UV-VIS spectrophotometer. Fluorescence spectra measurements were performed on a JASCO FP-6500 spectrofluorimeter equipped with a xenon discharge lamp, 1 cm quartz cells. All pH measurements were made with a Model pHS-3C pH meter (Shanghai, China). NMR spectra were recorded using a JOEL JNM-ECA300 spectrometer operated

* Corresponding author. Tel.: +86 10 62782485; fax: +86 10 62782485.
E-mail address: tongaj@mail.tsinghua.edu.cn (A. Tong).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.03.057



Scheme 1. Synthesis of compounds **1** and **2**.

at 300 MHz. ESI-MS spectra were obtained on an API3000 LC-MS spectrometer. Elemental analysis was carried out on a FLASH EA1112 elemental analyzer. Fluorescence lifetime was measured on a FLSP920 spectrophotometer (Edinburgh Instruments). All of the measurements were operated at room temperature at about 298 K.

2.3. Synthesis of ***1*** and ***2*** (Scheme 1)

2.3.1. Synthesis of salicylaldehyde acetic hydrazone (***1***) [29]

To a 25 mL flask were added 10 mL ethyl acetate (100 mmol) and 5 g hydrazine hydrate (85%, 85 mmol). The flask was allowed to stand in a 90 °C oil bath for 4 h. After cooled to room temperature, the excess ethyl acetate was removed under reduced pressure. 5.95 g colorless oil was obtained as rough product of acetic hydrazide.

0.74 g acetic hydrazide (about 10 mmol) prepared by the above method and 1.05 mL salicylaldehyde (1.17 g/mL, 10 mmol) were added to 20 mL absolute ethanol in a 50 mL flask. The mixture was stirred at room temperature for 30 min, and the resulting precipitate was filtered. After washing the solid for three times by 20 mL absolute ethanol and dried under reduced pressure, 0.96 g **1** (yield 54%) was obtained as white solid. ESI mass spectrometry: *m*/*z* 179.0 (20% $[M+H]^+$), 201.0 (80% $[M+Na]^+$); M^+ calculated 178.1. ^{1}H NMR (CD_3OD), δ (ppm): 8.25 (s, 1H), 7.36 (m, 1H), 7.28 (m, 1H), 6.89 (m, 2H), 2.07 (s, 3H). Elemental analysis: N 15.73%, C 60.62%, H 5.58%, calculated: $C_9H_{10}N_2O_2$, N 15.72%, C 60.66%, H 5.66%.

2.3.2. Synthesis of salicylaldehyde 4-pyren-1-yl-butyric hydrazone (***2***)

To a 50 mL flask were added 2.0 g 4-pyren-1-yl-butyric acid (7 mmol), 20 mL methanol and 2 mL concentrated H_2SO_4. The mixture was allowed to reflux for 2 h in a 70 °C oil bath. After cooled to room temperature, saturated Na_2CO_3 solution was added to neutralize the excess acid. Then the mixture was extracted two times by 20 mL dichloromethane. The organic phase was separated out and dried by anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. 1.7 g **3** (yield 87%) was obtained as brown oil. ^{1}H NMR (DMSO–d_6), δ (ppm): 8.50–7.80 (m, 9H, pyrene-H), 3.64 (s, 3H), 3.33 (t, 2H), 2.46 (t, 2H), 2.04 (m, 2H). ^{13}C NMR (DMSO–d_6), δ (ppm): 173.2, 136.1, 138.9, 130.4, 129.3, 128.1, 127.4 (2C, 127.39, 127.40), 127.2, 126.5, 126.0, 124.9, 124.9, 124.8, 124.2, 124.1, 123.3, 51.2, 32. 9, 31.8, 26.7.

1.5 g **3** (5 mmol), 10 mL hydrazine hydrate (85%) and 10 mL water were added to a 50 mL flask. The mixture was heated in an oil bath at 100 °C overnight. The resulting precipitate was filtered out and washed three times by 20 mL water. After dried under reduced pressure, 1.2 g **4** (yield 80%) was obtained as white solid. ^{1}H NMR (DMSO–d_6), δ (ppm): 9.10 (s, 1H), 8.50–7.80 (m, 9H, py-H), 4.32 (s, 2H), 3.34 (t, 2H), 2.27 (t, 2H), 2.07 (m, 2H). ^{13}C NMR (DMSO–d_6), δ (ppm): 171.5, 136.5, 130.9, 130.4, 129.3, 128.2, 127.4 (2C, 127.43, 127.44), 127.2, 126.5, 126.1, 124.9, 124.8, 124.2, 124.2, 123.5, 33.2, 33.1, 27.5.

0.8 g **4** (2.6 mmol), 0.312 mL salicylaldehyde (1.17 g/mL, 3 mmol), 20 mL glacial acetic acid and 10 mL water were added to a 100 mL flask. The mixture was heated in an oil bath at 80 °C for 50 min, and the resulting precipitate was filtered out. The solid was washed three times by 20 mL water and dried under reduced pressure. 0.720 g **2** (yield 67%) was obtained as offwhite solid. ESI mass spectrometry: *m*/*z* 407.2 (43% $[M+H]^+$), 429.2 (57% $[M+Na]^+$); M^+ calculated 406.2. ^{1}H NMR (DMSO–d_6), δ (ppm): 8.60–7.90 (m, 10H, py-H and NH), 7.02–6.80 (m, 2H, Benz-H), 6.38 (d, 1H, Benz-H), 6.11 (m, 1H, Benz-H), 3.36 (t, 2H), 2.40 (t, 2H), 2.10 (m, 2H). ^{13}C NMR (DMSO–d_6), δ (ppm): 170.5, 151.6, 138.2, 137.9, 133.1, 133.0, 131.4, 130.9, 129.6, 128.6, 128.0 (2C), 127.6, 126.9, 126.6, 125.4, 125.3, 125.2, 124.7, 124.6, 124.3, 122.0 (2C, 122.4, 122.3), 109.6, 33.2, 31.8, 29.9. Elemental analysis: N 6.94%, C 79.76%, H 5.57%, calculated: $C_{27}H_{22}N_2O_2$, N 6.89%, C 79.78%, H 5.46%.

2.4. Absorption and fluorescence measurements

Absorption and fluorescence titrations were performed by addition of small aliquots of one of **1** and **2** and metal ion working solutions to 3.0 mL buffered water/ethanol of different pH and ethanol fractions in a 1 cm quartz cell. After well mixed, the solutions were allowed to stand at ambient condition for 2 min, and absorption or fluorescence spectra were recorded.

Absorption and fluorescence spectra measurement of Zn^{2+} containing water samples was carried out by adding proper amount of one of **1** and **2**, buffer stock solution and ethanol to sample solutions, and then well mixed for 2 min before test.