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Substituent effect on fluorescence signaling of the naphthalene carbohydrazone based chemosensor: Its implication in the detection of Zn (II) ions and secondary sensing PP_i

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ABSTRACT

A series of naphthalene carbohydrazone based fluorescent chemosensors **L_1-L_4** were developed for the detection of Zn^{2+} by significant fluorescence enhancement. The binding ratio of the **L_1-Zn^{2+}** complex was determined by a Job's plot to be 1:1 and confirmed by ESI–MS studies. The corresponding of the **L_1-Zn^{2+}** ensemble was elucidated through X-ray crystallography along with spectroscopic studies. Furthermore, the **L_X-Zn^{2+} (X = 1–2)** complexes can specifically detect pyrophosphate (PP_i) in HEPES buffer solution through fluorescence quenching. The detection limit of **L_X-Zn^{2+} (X = 1–2)** for PP_i sensing were calculated to be as low as 2.54 ppb and 0.94 ppb, respectively. In addition, due to their good cell membrane permeability and low cytotoxicity, **L_1-L_4** have been applied to sequentially detect Zn^{2+} and PP_i in living cells. More importantly, the electronic effect of substitute groups on fluorescence signaling have been studied by substituent properties ranging from electron-donating to electron-withdrawing, which sets up a promising strategy for the rational design of fluorescent sensors.

1. Introduction

Zinc is the second most abundant transition metal ion in the human body after iron and is associated with proteins as a structural element or a catalytic factor [1]. It is believed to be related to many essential physiological activities in biological systems, such as neural signal transmission, enzyme regulation, and gene transcription [2,3]. Moreover, zinc deficiency leads to diminished cognition, immune dysfunction, and diarrhea, while the presence of excess free zinc in certain cells may be related to severe neurological disorders such as Alzheimer's and Parkinson's disease [4–6]. Above all, selective, sensitive and rapid detection of zinc ions in biological system is in great demand. Fluorescence-based detection systems are extremely popular in biochemical studies because of its high sensitivity, fast response time, low cost, signal simplification and great potential for imaging in live cells or tissues [7–10]. Though many fluorescence sensors based on small molecules for zinc ions in living systems have been developed in the last few years [11–19], and despite their strong potential in bioimaging of zinc [20]. However, most of them are not focused on the electronic effect of substitute groups for the influence of sensing performance.

Pyrophosphates ($P_2O_7^{4-}$, PP_i), the hydrolysate of adenosine triphosphate (ATP), are by-products of DNA replication (pyrophosphates are generated by DNA polymerases and catalyse a step in DNA elongation) and participate in energy transduction and several important metabolic processes that play irreplaceable roles in living systems [21,22]. PP_i concentration may be a useful biomarker for early cancer diagnosis and arthritic disease therapy [23]. Since Czarnik et al. introduced a polyamine-attached anthracene derivative to the PP_i-sensing field [24], numerous fluorescent chemosensor design strategies for detecting PP_i have been developed, which primarily involve hydrogen bonding [25,26], anion-π interactions [27], and metal-anion binding [28,29]. Zn(II) complexes, especially dinuclear complexes, show high selectivity and affinity towards PP_i in some cases and thus can be used to differentiate PP_i from other phosphate species [30,31]. Furthermore, Zn(II)-ligand coordinative interactions are stronger than hydrogen bonding and electrostatic interactions in physiological environments, rendering Zn(II) complexes a good platform for PP_i sensing [32–36]. Although there are some dinuclear zinc complex as fluorescent

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chemosensors for PP_i have been reported in recent years [37–40]; however, little efforts have been made about the electronic effect of substitute groups on the selectivity fluorescent sensor for PP_i, which remains a challenging and attractive target of fluorescence sensor performance study.

Herein, we have designed and synthesized a series of chemosensor **L_1-L_4** based on naphthalene carbohydrazone for Zn^{2+} and PP_i detection. The free sensors **L_1-L_4** were weakly fluorescent owing to the photo-induced electron transfe (PET) effect from the amine and the excited state intramolecular proton transfer (ESIPT) process. In the presence of Zn^{2+}, the sensors shows significant enhancement of fluorescence intensity which is because that the formation of 1:1 stoichiometric Zn(II) complex inhibit PET and ESIPT process. Moreover, the Zn (II)-sensor complex was applied to detect PP_i. The complexes **L_X-Zn^{2+} (X = 1–2)** showed good selectivity for the PP_i among various anions with a low detection limit even in the presence of other anions. To the best of our knowledge, the electronic effect of substitute groups for sensing of PP_i using dinuclear zinc complex has not been previously reported. Importantly, chemosensor **L_1-L_4** shows good cell membrane permeability and can be successfully applied to the imaging of Zn^{2+} and PP_i in living cells.

2. Materials and methods

2.1. Materials

p-Cresol, 4-methoxyphenol, ethyl-4-hydroxybenzoate, and 4-chlorophenol were purchased from Alfa Aesar (Tianjin, China) and used without further purification. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were purchased from Aladdin Industrial Corporation (Shanghai, China). Mouse fibroblast (L929) and human lung cancer (A549) cell lines were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum (FBS) were obtained from Invitrogen (Carlsbad, CA, USA). All other reagents were reagent grade and purchased from commercial sources.

2.2. Instruments

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 400 MHz and 100 MHz, respectively, using tetramethylsilane (TMS) as the internal standard. Mass spectrometry was performed on a Bruker micrOTOF-Q II. pH values were determined with a Sartorius PB–21 pH meter. Ultraviolet-visible (UV–vis) absorption spectra in the region from 200 to 500 nm were recorded on a Shimadzu UV-1700 spectrophotometer using cuvettes with a 1 cm path length. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer equipped with a xenon lamp. Fluorescence microscopy imaging experiments were performed on an Olympus FV 1000 confocal laser scanning microscope (Japan). Cell viability assays were conducted using a Spectra Max 190 microplate reader. Melting points were determined on an X-4 Digital Vision MP Instrument.

2.3. UV–vis and fluorescence measurements

Stock solutions of **L_1-L_4** (2×10^{-3} M) were prepared in absolute DMSO and diluted to 20 μM with DMSO/aqueous HEPES buffer (10 mM, pH = 7.4, 2:8 v/v). All emission spectra were recorded in the wavelength range of 450–700 nm with an excitation wavelength of 440 nm. In addition, the slit-widths for excitation and emission were 5.0 nm, and the PMT voltage was 700 V.

2.4. Recognition performance studies

Stock solutions of **L_1-L_4** (2×10^{-4} M) were prepared in DMSO and diluted to 20 μM with DMSO/HEPES buffer (10 mM, pH = 7.4, 2:8 v/v). The 2×10^{-3} M stock solutions of metal ions (Na^+, K^+, Ag^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Hg^{2+}, and Pb^{2+}) were prepared in distilled water using their chloride salts, except for the Ag^+ stock solution, which was prepared using $AgNO_3$. The 2×10^{-3} M stock solutions of anions (ATP, ADP, AMP, PP_i, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, CH_3COO^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, F^-, Cl^-, and I^-) were prepared in distilled water using their sodium salts. All experiments were performed in a mixed solvent of DMSO/HEPES buffer (10 mM, pH = 7.4, 2:8 v/v).

During the titration experiments, different amounts of Zn^{2+} and 0.10 mL of 200 μM probe **L_1-L_4** were mixed and diluted to a volume of 10 mL in volumetric tubes using DMSO/aqueous HEPES buffer (10 mM, pH = 7.4, 2:8 v/v). Different amounts of PP_i and 0.10 mL of 200 μM **L_1-Zn^{2+}** were mixed and diluted to 10 mL in volumetric tubes using DMSO/aqueous HEPES buffer (10 mM, pH = 7.4, 2:8 v/v). For the selectivity experiments, 0.5 mL of cation or anion solution (2 mM) and 1 mL of probe solution (200 μM) were added to a 10 mL volumetric tube and mixed.

2.5. Synthesis of L_1-L_4

The design strategy and synthetic routes for **L_1-L_4** are shown in Scheme 1; the intermediate **A_1-A_4** were prepared as described previously [41]. NMR, ESI–MS, and crystallographic data were obtained to characterize the geometric structure of each compound. Characterization spectra of all compounds are provided in Supplementary Material.

Synthesis of **L_1**: N-(Aminomethyl)-2-naphthamide (0.372 g, 2 mmol) was slowly added to a methanolic solution (15 mL) containing 2,6-diformyl-4-methylphenol (**A1**: 0.164 g, 1 mmol), and then the mixture was then stirred at 50 °C for 4 h. Next, the solution was concentrated under reduced pressure to form a yellow turbid liquid, and the precipitate was filtered, washed three times with cold methanol and recrystallized from ethanol to obtain a yellow solid (yield: 93%). m.p. 110–111 °C, ^{1}H NMR (400 MHz, DMSO-*d*6), δ (ppm): 12.41 (1H, s), 12.35 (2H, s), 8.79 (2H, s), 8.60 (2H, s), 8.08 (8H, *J* = 23.3, 7.6 Hz, dd), 7.64 (6H, *J* = 11.6, d), 2.36 (3H, s); ^{13}C NMR (100 MHz, DMSO-*d*6), δ (ppm): 163.7, 155.5, 146.9, 135.1, 132.9, 131.1, 130.9, 129.6, 128.9, 128.7, 128.4, 127.6, 124.9, 120.5, 20.6. ESI–MS: *m/z* calc'd for $C_{31}H_{24}N_4O_3$: 500.1848; found, 523.1731 $[M + Na]^+$.

Synthesis of **L_2**: N-(Aminomethyl)-2-naphthamide (0.372 g, 2 mmol) was slowly added to a methanolic solution (15 mL) containing 2,6-diformyl-4-methoxyphenol (**A_2**: 0.180 g, 1 mmol), and the mixture was then stirred at 50 °C for 4 h. Next, the solution was concentrated under reduced pressure to afford a yellow turbid liquid, and the precipitate was filtered, washed three times with cold methanol and recrystallized from ethanol to obtain an orange solid (yield: 86%). m.p. 110–113 °C, ^{1}H NMR (400 MHz, DMSO-*d*6), δ (ppm): 12.40 (2H, s), 12.18 (1H, s), 8.81 (2H, s), 8.60 (2H, s), 8.12-8.08 (4H, m), 8.03 (4H, *J* = 7.0 Hz, d), 7.69-7.64 (4H, m), 7.39 (2H, s), 3.84 (3H, s). ^{13}C NMR (100 MHz, DMSO-*d*6), δ (ppm): 163.5, 152.5, 146.4, 134.9, 132.5, 130.6, 129.5, 129.1, 128.1, 127.5, 124.8, 121.3, 115.5, 19.0. ESI–MS: *m/z* calc'd for $C_{31}H_{24}N_4O_4$: 516.1798; found, 539.1692 $[M + Na]^+$.

Synthesis of **L_3**: N-(Aminomethyl)-2-naphthamide (0.372 g, 2 mmol) was slowly added to a methanolic solution (15 mL) containing ethyl-3,5-diformyl-4-hydroxybenzoate (**A_3**: 0.222 g, 1 mmol), and the mixture was then stirred at 50 °C for 4 h. Next, the solution was concentrated under reduced pressure to form a yellow turbid liquid, and the precipitate was filtered, washed three times with cold methanol and recrystallized from ethanol to obtain a light yellow solid (yield: 88%). m.p. 108–110 °C, ^{1}H NMR (400 MHz, DMSO-*d*6), δ (ppm): 13.51 (1H, s), 12.55 (2H, s), 8.89 (2H, s), 8.64 (2H, s), 8.42 (2H, s), 8.12 (4H, *J* = 8.7 Hz, d), 8.06 (4H, *J* = 7.4 Hz, d), 7.69 (4H, *J* = 7.2 Hz, t), 4.42