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An active fluorescent probe based on aggregation-induced emission for intracellular bioimaging of Zn^{2+} and tracking of interactions with single-stranded DNA

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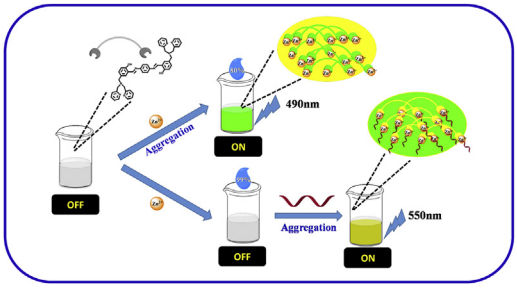
HIGHLIGHTS

- A novel dual-sensing fluorescence probe based AIE characterization was designed and synthesized.
- The probe L exhibited a detection limit as low as 3.8 nM for Zn^{2+} in a wide linear range.
- The probe L has low cytotoxicity and good cell-permeability, and has been applied for intracellular imaging of Zn^{2+}.
- The probe L toward Zn^{2+} can be used to detect qualitatively low levels of ssDNA.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel dual-sensing fluorescence probe L was designed and synthesized for highly selective and sensitive detection of Zn^{2+} and DNA. The probe L achieved a detection limit of 3.8 nM for Zn^{2+}, which is lower than the acceptable level of Zn^{2+} in living cells. The probe L displayed high selectivity toward Zn^{2+} over other interference metal ions and amino acids. Moreover, the probe L displayed low cytotoxicity and good cell permeability, indicating its potential for detecting and bio-imaging of Zn^{2+}. In addition, the probe L-Zn^{2+} exhibited enhanced fluorescence signal for DNA detection through the metal–coordination interaction between Zn^{2+} and DNA. The enhanced signal is higher than that of the classical ethidium bromide probe. The experiments in aqueous media verified the feasibility of applying probe L in real samples.

1. Introduction

As the second most abundant transition metal, zinc (2–4 g) is distributed as an intrinsic ingredient throughout the human body to maintain life [1,2]. Zn^{2+} is involved in many important biological processes, including cell growth, cell division, cell transport [3], gene expression [4–6], cell apoptosis [7], neural signal transmission [8], and mammalian reproduction [9,10]. Previous studies reported that approximately 20 μM Zn^{2+} is distributed in cells [11]. When zinc is not metabolized in equilibrium, it may cause neurological disorders and other severe diseases, such as Alzheimer's disease [12,13], immune deficiency [14], and epilepsy [15]. Zn^{2+} must be monitored in living cells to elucidate its biological function and other related mechanisms. Considering the physiological

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significance of Zn^{2+}, researchers have focused on developing methods for highly sensitive and selective detection and monitoring of Zn^{2+} in biological systems [16]. Fluorescence techniques are the most rapid and selective separation method that can monitor and control the concentration ranges of Zn^{2+} in organisms; this method can also be used to observe the subcellular distribution of Zn^{2+} in living cells [17–24].

Fundamental physiological studies [25,26] have focused on developing sensitive and cost-effective fluorescent probes to monitor and detect nucleic acids. Nucleic acid sequences, which represent genetic information, can clarify and explain the physiological functions of these molecules in biological systems [27–32]. Given their poor selectivity for DNA, typical nucleic acid probes distinguish and identify nucleic acids on the basis of intercalation [33], groove binding [34], electrostatic interaction [35], and hydrogen bonds [36]. Many fluorescent probes based on intercalation or groove binding have been designed and utilized to detect double-stranded DNA [37,38]. However, single-stranded DNA (ssDNA) contains unstable secondary structures, for which many existing probes generally display weak fluorescence intensity. Several researchers have designed various fluorescent molecules [39–42] (e.g., pyrene, polythiophene, and tetraphenylethenes) as ssDNA probes based on electrostatic interactions and hydrogen bonds. However, electrolytes in aqueous medium can interfere with electrostatic interactions and hydrogen bonds, thereby restricting the practical applications of these probes. Therefore, novel probes must be developed for selective sensing of ssDNA in aqueous media.

In recent years, many zinc complexes have been prepared as high-sensitivity DNA probes for selective detection of ssDNA in aqueous media [43–46]. Metal–coordination interactions are more efficient than hydrogen bonds and electrostatic interactions in aqueous solutions. Compared with homologous probes based on electrostatic interactions, metal complexes display higher sensitivity for detection of ssDNA [47–50]. However, limited works have explored fluorescent probes for simultaneous dual-sensing of nucleic acids and metal ions.

Fluorescent probes with aggregation-induced emission (AIE) have attracted considerable attention. The weak fluorescence emission of these probes in dilute solutions can be significantly enhanced by aggregation because of the interaction between the probe and the target in dilute solutions or in solid states. In addition, an excited-state intramolecular proton transfer (ESIPT) mechanism was further introduced to AIE fluorogens, which allowed for the development of light-up probes with sharp imaging contrast, high signal-to-noise ratios, and extremely large Stokes shifts [51,52]. Therefore, AIE active probes are considered outstanding candidates for quantitative detection of target materials [53–57]. In addition, fluorescence sensing systems based on AIE can respond to other competing species that can adjust the interaction between the probe and the target, thereby enabling the probe to achieve dual sensing.

Considering the aforementioned requirements, we successfully designed and synthesized an AIE active probe L (Scheme S1), which displays a selective fluorescence turn-on response toward Zn^{2+} in mixed aqueous media. The AIE properties also captured the subsequent interaction of the probe L-Zn^{2+} ensemble with ssDNA by adjusting the conditions of the aqueous medium. Thus, once the probe L-Zn^{2+} binds to ssDNA (Scheme 1), the AIE active probe L units are expected to aggregate because of the interaction between Zn^{2+} and the DNA chain, resulting in enhanced fluorescence emission.

2. Materials and methods

2.1. General information

^1H NMR and ^{13}C NMR studies were performed on AVANCE III HD 600 MHz NMR spectrometer. Fourier transform infrared (FT-IR) spectra were obtained using a Nicolet 380 FT-IR spectrometer within the 4000–400 cm^{-1} wavelength range. Fluorescence spectra were obtained by utilizing CaryEclipse spectrophotometer (USA). UV–vis absorption spectra were recorded using a UV-4100 spectrophotometer (Shimadzu, Japan). Cell imaging was performed with a confocal microscope (Olympus FV 1000, Tokyo, Japan). Circular dichroism was measured by using a Circular Dichroism Spectrometer (J-815, Japan). All reagents and solvents were commercially available and used without further purification. Double-distilled water was used throughout the experiments. ssDNA was purchased from SangonBiotech (Shanghai) Co., Ltd. This study utilized the DNA sequence 5′-TTAGCTTATGCGTTGGTTGTAGATT-3'.

2.2. Synthesis and characterization

2.2.1. 5-(Chloromethyl)-2-hydroxybenzaldehyde **1**

5-(Chloromethyl)-2-hydroxybenzaldehyde **1** was prepared and purified according to a previously reported method [58].

2.2.2. Synthesis of 5-((bis(pyridin-2-ylmethyl)amino)methyl)-2-hydroxybenzaldehyde **2**

Compound **1** (680 mg, 4.0 mmol) and sodium carbonate (624 mg, 4.0 mmol) were dissolved in anhydrous DMF (20 mL), then added with di-(2-picolyl) amine (796 mg, 4.0 mmol). The mixture was stirred at 60 °C under N_2 for 12 h. After cooling to room temperature, the reaction mixture was extracted with dichloromethane (50 mL × 3). The three combined organic fractions were dried under anhydrous Na_2SO_4 and then concentrated. The residue was further purified by column chromatography to yield a milky oil as compound **2** (932 mg, 70% yield). ^1H NMR ($CDCl_3$, 600 MHz): δ 10.97 (s, 1H), 9.89 (s, 1H), 8.55 (d, 2H), 7.68–7.50 (m, 6H), 7.16 (d, 2H), 6.93 (d, 1H), 3.83 (s, 4H), 3.77 (s, 2H). ^{13}C NMR ($CDCl_3$, 600 MHz): 196.5, 160.8, 150.0, 137.8, 136.6, 133.9, 123.1, 122.2, 120.2, 117.6, 59.8, 57.3. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{20}H_{20}N_3O_2$: 334.1550; found: 334.1546.

2.2.3. Synthesis of compound **3** (probe L)

Compound **2** (664 mg, 2.0 mmol) was dissolved in absolute ethanol (10 mL), which was then added with p-phenylenediamine (108 mg, 1.0 mmol). The mixture was refluxed for 4 h. After being cooled to room temperature, the solvent was removed and dried under vacuum to an orange solid as probe L (628 mg, 95% yield). ^1H NMR (DMSO-d6, 600 MHz): δ 13.17 (s, 2H), 8.67 (s, 2H), 8.53 (d, 4H), 7.68 (t, 4H), 7.56 (t, 4H), 7.44–7.19 (m, 8H), 6.97 (d, 2H), 6.71 (d, 2H), 3.70 (s, 8H), 3.66 (s, 4H). ^{13}C NMR (DMSO-d6, 600 MHz): 166.1, 160.7, 160.2, 149.6, 138.9, 135.5, 134.2, 131.1, 125.1, 124.0, 118.9, 118.0, 60.9, 59.1. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{47}H_{46}N_8O_2$: 739.3190; found: 739.3212.

2.3. Intracellular imaging of Zn^{2+}

HeLa cells were provided by NIH. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 100 U/mL penicillin, 10% fetal bovine serum (FBS), and 100 mg/mL streptomycin under 5% CO_2 at 37 °C. The HeLa cells were washed thrice with PBS

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