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A novel 'turn-on' fluorescence probe with aggregation-induced emission for the selective detection and bioimaging of Hg^{2+} in live cells

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ABSTRACT

A novel 'turn on' fluorescent probe with aggregation-induced emission was designed, prepared, characterized and applied to fluorescence imaging of Hg^{2+} in live HeLa cells. Unique recognition mechanism of the probe toward Hg^{2+} has been determined by UV/vis, fluorescence spectra, SEM, mass spectra and ^1H NMR studies, respectively. The probe displayed higher properties such as, stability to pH, fast-response time with Hg^{2+}, a lower detection limit, stronger antijamming capability and better imaging in live cells compared with other probes. More importantly, to further exhibited the practicability of the probe in biological system, the probe was applied to detect Hg^{2+} in live HeLa cells. The fluorescence images for detection of intracellular Hg^{2+} at different concentrations have been performed. The superior properties of the probe make it of great potential use *in vitro* or *in vivo* sensing some species of biologically studies.

1. Introduction

Mercury ion (Hg^{2+}) is considered as the most toxic and dangerous contamination among these heavy and transition metal ions. Mercury exists in the form of inorganic salt or organic mercury compound in food chain. It has a high capability of coordination with sulfhydryl groups in biological systems and can be converted into organic mercury by contaminant in the environments [1–3], and its harmful effects is long and lasting exist in biological systems, resulting in many diseases and even death [4–10]. At present, Mercury detection in various samples, especially in biological systems, has been a critical issue [11]. Therefore, development of highly sensitive and selective systems for *in vivo* or *in vitro* monitoring of mercury in biological systems was attracted academic researchers interest. Most reported systems for detection of mercury are based on conjugated polymers [12], DNAzymes [13], proteins [14], nanoparticles [15,16] and so on. These detection systems have the advantages of their relatively low detection limit and are suitable for detecting trace mercury. Yet most of them have one of the drawbacks or limitations, such as complicated synthetic, slow response time, the interference in the detection process and cytotoxicity [17,18]. Consequently, it is highly appealing to develop simple, low-cost and rapid method with high sensitivity and selectivity for mercury detecting in biological molecules. Molecular fluorescence is promising in biological and analytical fields for mercury detection [19].

At present, many fluorescent probes for mercury detection have been reported and developed [20–25], due to advantages of their sensitivity, selectivity, simplicity, low price, biocompatibility and so on. Nevertheless, the fluorescence molecules, usually tend to aggregate in solution of high concentration or coordinating with heavy metal ions [26,27], resulting in the aggregation-caused quenching (ACQ). It will become more severe especially when imaging low-abundant molecular species in biological systems, since the fluorescence signals could not be enhanced by increasing the fluorophore concentrations [28–30]. Most reported fluorescent sensors for mercury detection were designed based on fluorescence quenching by electron or energy transfer mechanism [31]. And turn-off mode was not sensitive as a reaction signal that the quenching could easily experience interference by many external factors [32–34]. Although some fluorescence "turn-on" sensors for mercury detection began to be investigated and displayed certain application recently due to the high reaction signal and sensitivity, design and synthesis new fluorescent sensors for selectively and sensitively detecting mercury by a turn-on response is still a critical problem.

In recent years, a novel method for the detection of metal ions and biomolecules based on the unusual fluorescence properties of

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aggregation-induced emission (AIE) has been recommended which was discovered by Tang et al. in 2001 [35,36]. The simple design and fluorescence turn-on feature of the molecular AIE bioprobes offer direct visualization of specific analytes and biological processes with higher sensitivity and better accuracy than traditional fluorescence turn-off probes [37,38]. Many AIE active fluorescent probes have been reported for detection various materials [39–44], such as metal ions, proteins, DNA, polysaccharide, cancer cells and so on. Recently, Yang et al. group reported three AIE/AIEE type "turn-on" fluorescent Hg^{2+} sensors by combined Schiff base or coumarin with α-cyanostilbene [45,46]. However, as far as we know, there are lesser reports on fluorescent "turn-on" probes for mercury detection in live cells based AIE/AIEE characterization.

In this work, we designed and synthesized an effective fluorescence probe of AIE properties based on Schiff base derivatives, which exhibited excellent fluorescence detection of Hg^{2+} in live HeLa cells by "turn-on" response (Scheme 1). The AIE properties of the probe and the probe for Hg^{2+} detection ability were studied. In addition, the probe exhibited good biocompatibility, low cytotoxicity and it also has been used for detecting Hg^{2+} in live cells with satisfactory results. The probe is promising an excellent AIE compound and effective fluorescence probe for the detection of Hg^{2+} in biological samples.

2. Experimental section

2.1. Reagents and materials

All reagents and solvents used in the experiment were commercially available and used without further purification. The reagents Triphenylamine, Salicylaldehyde and 1,2-dibromoethane for the synthesis of the probe were purchased from Aladdin (Shanghai, China). All other chemicals, including $Hg(Cl)_2$, $Cr(NO_3)_3$, $Cu(NO_3)_2$, $Co(NO_3)_3$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Fe(NO_3)_3$, $Mg(NO_3)_2$, $Ni(NO_3)_2$, $AgNO_3$, $Cd(NO_3)_2$, $Mn(NO_3)_2$, and so on, were of analytical reagent grade and were used as received without further purification. Double-distilled water was used in the experimental process. A stock solution of the probe (1.0×10^{-3} M) was prepared. This stock solution was used for all fluorescence titration measurements. But in cell imaging test, the probe was prepared in DMSO. UV–vis absorption spectra measurements were recorded using a UV-4100 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were measured with CaryEclipse spectrophotometers (America). ^{1}H NMR and ^{13}C NMR measurements were performed on AVANCE III HD 600 MHz NMR spectrometer by using dimethylsulfoxide (DMSO)-d_6 as a solvent. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 380 FT-IR spectrometer in the 4000–400 cm^{-1} wavelength range. The pH of the stock solutions was measured using a pH Meter (Jinpeng Analytical Instruments Co. Ltd, China). Cell imaging was performed with a confocal microscope (Olympus FV 1000, Tokyo, Japan).

2.2. Synthesis of the probe

Compound **1** and **2** were synthesized and purified by the previously reported procedure [47,48].

The probe was synthesized by the following procedure. The compound 2 (0.27 g, 1 mmol) in methanol (10 mL) was heated until dissolved completely, and the solution was then cooled to room temperature. To the solution was slowly added a solution of excess compound **1** (1.18 g, 4 mmol) in 30 mL methanol, then the mixture was stirred for 20 h at reflux. A yellow mixture was obtained gradually. The precipitate was filtered and washed four times with 30 mL ethanol. After drying under reduced pressure, the yield of probe was 0.61 g (79%). FT-IR (KBr, cm^{-1}): 3070, 2917, 2854, 1631, 1589, 1513, 1492, 1436, 1274, 1052, 833, 752, 696. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm): 8.90 (s, 2H), 8.08 (d, 2H), 7.96 (d, 2H), 7.68 (d, 4H), 7.54 (d, 2H), 7.46 (t, 2H), 7.16 (d, 4H), 7.13 (t, 4H), 6.95 (d, 4H), 6.82 (m, 4H), 6.67 (d, 2H), 4.56 (s, 4H). ^{13}C NMR ($CDCl_3$, 600 MHz): δ (ppm): 162.6, 161.5, 154.8, 147.9, 134.6, 131.3, 129.1, 127.7, 126.2, 125.1, 123.5, 121.3, 116.6, 113.9, 67.38. ESI-mass (*m*/*z*): calcd for $C_{52}H_{42}N_4O_2$ $[M+H]^+$, 755.35; found, 755.33.

2.3. Analytical procedures

To evaluate the probe for Hg^{2+} detection ability, 20 μL of Hg^{2+} with different concentrations was added to the solution containing 10 mM PBS buffer and 10 μM probe; then, the mixture was mixed and 8 min later, the fluorescence spectra was recorded. While for experiments about the absorption spectra, the concentration of the probe was 10 μM to obtain a relative intensive absorption signal and the same procedure as that forementioned was performed.

To investigate the selectivity of the probe for Hg^{2+}, different other metal ions, including Ag^+, Cd^{2+}, Co^{2+}, Pb^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Fe^{3+} of identical concentrations, were added into the sensing solution and the fluorescence spectra was recorded.

All experiments were repeated three times and all experiments were carried out at room temperature.

2.4. Cell culture, incubation and imaging

The probe sensing system is used for *in vitro* imaging of mercury in live Hela cells. HeLa cells were obtained from 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. After 24 h of incubation in DMEM, 20 μL of the probe (10 μM) was incubated with 980 μL of a cell solution in DMEM for 10 min at 37 °C, and the cells were washed for three times with PBS buffer solution (10 mM, pH 7.4). And then the fluorescence imaging of cells was performed on a confocal microscope. Afterward, 1.0 mL of different concentrations of Hg^{2+} solution (5 and 10 μM) in PBS buffer (10 mM, pH 7.4) was added to the above cells for incubation for another 10 min. Then, the cells were washed for three times with PBS buffer solution (10 mM, pH 7.4), and fluorescence imaging of the cells was carried out. The excitation and emission wavelength were at 365 nm and 450 (±10) nm, respectively.

2.5. Cell cytotoxic test

The cytotoxic test of the probe was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. After 24 h incubation in DMEM at 37 °C, different concentrations (0, 20, 40, 60, 80, 100 μM) of the probe were deal with the cell. The cells were further cultured for additional 12 h. Soon after, 10 μL of MTT was added and further incubated for another 4 h. Then, the supernatant was discarded, 150 μL of DMSO was added to well, and the plate was shaken for 15 min to dissolve the insoluble MTT product. The absorbance values of the wells were tested using a micro-plate reader. The cell viability was calculated using the equation $VR = A/A_0 \times 100\%$, in which A is the absorbance of the probe treated cells and A_0 is the absorbance from cells without treatment by the probe. Each measurement was performed five times.