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Short communication

An anthraquinone-based "turn-on" fluorescence probe for Hg^{2+} detection and its application in cell imaging



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ARTICLE INFO	A B S T R A C T
Keywords: Turn-on Fluorescence Desulfurization Hg ²⁺	A new highly sensitive fluorescence "turn-on" probe (HgP1) based on anthraquinone derivatives for the detection of Hg^{2+} has been designed and developed. Based on desulfurization effect, probe HgP1 shows high selectivity towards Hg^{2+} in CH ₃ OH/HEPES (10 mM, pH = 7.4, 1/1, v/v) solution over other analytes. The detection limit for Hg^{2+} is 8.2 nM. In addition, the probe shows good biocompatibility and has been successfully used in living cell imaging.

1. Introduction

Mercury is one of common pollutants in organisms and the environment. At room temperature, mercury exists in liquid form and is easy to volatile. Mercury vapor and mercury compounds are highly toxic, which cause serious pollution to the ecological environment [1]. Mercury ion ($\rm Hg^{2+}$) pollution in the environment has caused serious harm to animals, plants and human health [2–3]. For example, some genetic in plants and animals will be changed with excess mercury ions. As a typical Lewis acid, mercury ions have a strong thiophilic property, and will react with proteins or enzymes that contain sulfur in living organisms, causing a range of diseases, including excessive mercury ions that can lead to irreversible DNA damage, pulmonary edema, kidney failure and many types of autism [4–9]. As the hypertoxicity of mercury ions, the rapid and real-time detection of the content of mercury ions in the environment is of great importance, and it is particularly urgent need in food safety and biomedical fields [10].

Because of its obvious advantages of low cost, simple instrument operation, low detection limit and real-time monitoring, fluorescence probe detection of metal ions has attracted widely attention in recent years [11–20]. Among various of fluorescent probes, reactive probes show high selectivity to target analytes due to the irreversible chemical reaction with the target analytes, which can reduce the detection error and improve the detection accuracy. Therefore, it has a good application value for heavy metal detection in complex systems [21–26]. For mercury ion recognition, the main strategy is based on its strong thiophilic property [27–29]. In the desulfurization progress between mercury ions and sulfur atoms, the fluorescence intensity will be changed as the structure of fluorescent probe changed. Anthraquinone derivatives have the advantages of easy synthesis, large stoke shift, good photostability, and can form excited intramolecular charge transfer (ICT), which are suitable precursors for designing new fluorescent probes. Based on the strong sthiophilic property of Hg^{2+} , a fluorescence "turn-on" probe which containing thioacetals as the reactive site for Hg^{2+} and anthraquinone derivatives as fluorophore was designed. Besides of good selectivity and sensitivity, the probe overcome the interference of common metal cations and anions, and can effectively identify Hg^{2+} in complex systems. In addition, the probe showed low biological toxicity and had been successfully applied to detect exogenous Hg^{2+} in living cells.

2. Experimental

2.1. Apparatus and materials

NMR data was collected by Bruker AVII-400 instrument. High resolution mass spectrometry data were recorded by an HPLC Q-Exactive HR-MS spectroscopy (Thermo). The fluorescence spectra were recorded by Hitachi F-7000 fluorescence spectrometer. Fluorescence images experiments were performed by an Olympus FV-3000 confocal

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microscope.

The solvents and reagents used in the experiment are analytically pure, purchased from commercial companies and used directly. The metal ions used in the analysis experiment are corresponding chloride or nitrate salts, dissolved in distilled water to prepare a 10 mM stock solution. The probe **HgP1** was dissolved in MeOH to prepare 1 mM stock solution.

2.2. Synthesis

Compound **QCHO** was synthesized by referring to the known literature [30]. The detail synthetic steps of probe **HgP1** are as follows:

Compound QCHO (352 mg, 1 mmol) and p-toluenesulfonic acid (17.2 mg, 0.10 mmol) were dissolved in 25 mL anhydrous dichloromethane solution, and ethyl mercaptan (497 mg, 8 mmol) was added to the above solution. And the reaction solution was stirred at room temperature for about 15 h under N2 atmosphere. After the reaction is complete, 30 mL saturated NaCl solution was added to the above solution. Then the organic solvent was separated, and dried over anhydrous Na₂SO₄. The crude product was purified by silica gel chromatography (petroleum/ ethyl acetate = 15:1, v/v) to get probe HgP1 as a brown solid (343.5 mg, 75%). ¹H NMR (CDCl₃, 400 MHz) δ 1.15 (t, J = 7.0 Hz, 6H), 2.62 (m, 4H), 5.01 (s, 1H), 7.67 (d, J = 7.6 Hz, 2H), 7.80 (s, 2H), 8.12 (t, J = 9.2 Hz, 3H), 8.23 (t, J = 7.8 Hz, 2H), 8.32 (s, 1H), 11.28 (s, 1H); 13 C NMR (CDCl₃, 100 MHz) δ 185.19, 182.61, 156.19, 149.51, 144.34, 134.48, 133.98, 133.81, 133.33, 133.18, 128.72, 128.67, 128.07, 127.62, 127.33, 126.52, 125.73, 122.06, 118.06, 52.16, 26.34, 14.32; HR-ESI-MS calcd for [M + H⁺]⁺ (C₂₆H₂₃N₂O₂S₂): 459.1201, found 459.1188 [M + H⁺].

3. Results and analysis

Probe **HgP1** was synthesized in a simple step, and its chemical structure was verified by ¹H NMR, ¹³C NMR and HRMS (Fig. S1-S3). Probe **HgP1** showed good solubility in common organic solvents. By referring to the previous experimental methods [31], the best recognition system for Hg²⁺ is CH₃OH/HEPES(10 mM, pH = 7.4, 1/1, v/v) solution (Fig. S4).

3.1. Fluorescence spectra

Specific selectivity is an important factor to evaluate the efficiency of fluorescent probe molecules. Firstly, the selectivity of probe **HgP1** towards various metal ions was investigated by fluorescence spectrometer. As shown in Fig. 1, the fluorescence emission intensity of the single

Fig. 1. The fluorescence emission spectra of probe HgP1 (10 μ M) towards different metal ions (100 μ M) in CH₃OH/HEPES(10 mM, pH = 7.4, 1/1, v/v) solution (λ_{ex} = 390 nm).

probe HgP1 in CH₃OH/HEPES(10 mM, pH = 7.4, 1/1, v/v) solution system was weak between 400 nm and 700 nm, and the fluorescence emission of the solution system was significantly enhanced when Hg²⁺ (10 eq.) was added. However, when 10 eq. of various common metal ions (K⁺, Na⁺, Li⁺, Ca²⁺, Mg²⁺, Ba²⁺, Zn²⁺, Sn²⁺, Sn⁴⁺, Fe²⁺, Mn²⁺, Pb²⁺, Cu²⁺, Co²⁺, Fe³⁺, Cr³⁺, Ag⁺, Ni²⁺, Cd²⁺) were added, the fluorescence emission of the solution system did not change significantly. The above experimental results showed that the probe has good selectivity to Hg²⁺.

In order to test potential competition of probe **HgP1** towards Hg^{2+} detection, the fluorescence emission spectra of the probe recognizes Hg^{2+} were measured in the presence of common metal ions and anions. As shown in Fig. 2, in the presence of other metal ions or anions, the fluorescence emission intensity (at 544 nm) of the probe for Hg^{2+} recognition is almost the same as that of Hg^{2+} recognition alone. The results showed that the probe can be used to identify Hg^{2+} under complex conditions, which improves the practical application value of the probe.

Good detection limit is one of the criteria to check whether a probe molecule has application value. Fluorescence spectrometer was used to determine the Hg^{2+} detection limit in CH₃OH/HEPES (10 mM, pH = 7.4, 1/1, v/v) solution. As shown in Fig. 3A, the single probe (10 μ M) exhibits weak fluorescence emission in the CH₃OH/HEPES (10 mM, pH = 7.4, 1/1, v/v) solution. With the continuous addition of Hg²⁺, the fluorescence emission intensity of the solution system increases continuously, indicating that probe HgP1 is a fluorescence "turn-on" probe for the detection of Hg^{2+} . It should be noted that the fluorescence emission intensity (at 544 nm) of the solution system has a good linear relationship ($R^2 = 0.990$) with the concentration of Hg²⁺ in the range of $0-1.0 \ \mu\text{M}$ (Fig. 3B). According to the calculation of IUPAC (3sd/k), the detection limit of Hg^{2+} by this probe is estimated to 8.2 nM [32], which can meet the national requirement of limiting the content of Hg²⁺ in food and water, indicating that this probe HgP1 has great application value in the quality and safety of agricultural products [33].

In order to further evaluate the effect of different pH of buffer solution on the detection of Hg^{2+} by probe HgP1, fluorescence titration experiments of buffer solutions with different pH values were carried out. As shown in the Fig. 4, the fluorescence emission intensity (at 544 nm) of the single probe $(10 \,\mu\text{M})$ is very low and stable between pH 4 and 9. In the presence of Hg^{2+} (100 $\mu M), the solution system exhibits strong$ fluorescence emission at pH 4–8, indicating that the probe can be used to identify Hg²⁺ under physiological conditions. The result of kinetic experiments shows that the fluorescence emission of the solution reaches the maximum value in about 400 s (Fig. S5), which further indicates that the probe has a rapid recognition effect on Hg^{2+} . Comparing with the fluorescent probes based on desulfurization reaction mechanism for the detection of Hg^{2+} listed in Table S1, the probe HgP1 demonstrates moderate advantages in the aspects of detection limit, recognition time, recognition system and application field, which provides a new choice for the detection of Hg^{2+} .

3.2. Mechanism

The above results showed that the probe **HgP1** has good recognition performance for Hg^{2+} , and we further studied the mechanism of detection. Based on the addition of EDTA, the reversible recognition experiment of Hg^{2+} was investigated (Fig. S6). When excess EDTA was added to the **HgP1**-Hg²⁺ solution, the fluorescence emission spectrum was almost the same as the **HgP1**-Hg²⁺ solution, which indicates that the coordination of **HgP1** with Hg²⁺ is chemically nonreversible. ¹H NMR titration experiment was conducted on probe **HgP1** with different amounts of Hg²⁺ in DMSO-*d*₆ (shown in Fig. S7). A new peak at 9.65 ppm (H₁) which assigned to the proton on the –CHO signal appeared and increased, along with the amounts of Hg²⁺ increasing. Meanwhile, the peak at 5.30 ppm (H₁), assigned to methine proton of the thioacetal group in probe **HgP1** were gradually decreased and finally disappeared.





Fig. 2. Fluorescence emission (at 544 nm) of Hg²⁺ (100 μ M) recognized by the probe HgP1 (10 μ M) in the presence of metal ions (100 μ M, A) and anions (100 μ M, B) ($\lambda_{ex} = 390$ nm).



Fig. 3. (A) The fluorescence emission spectra of probe HgP1 (10 μ M) with different concentrations of Hg²⁺ (0–10 eq.), (B) the linear fluorescence emission responses (at 544 nm) of Hg²⁺ (0–1.0 μ M) (λ_{ex} = 390 nm).



Fig. 4. Fluorescence response responses (at 544 nm) of probe HgP1 (10 μ M) with/without Hg²⁺ (100 μ M) under different pH values in CH₃OH/HEPES (10 mM, 1/1, v/v) solution ($\lambda_{ex} = 390$ nm).

Based on the above experimental results of fluorescence spectra and previous work [34–40], we proposed a receivable mechanism. As shown in Scheme 2, based on the strong thiophilic affinity of Hg^{2+} , Hg^{2+} can react with dithioacetal in the probe structure to release aldehyde group, and then the intramolecular charge transfer (ICT) of the anthraquinone was enhanced, resulting in fluorescence recovery. The high resolution mass spectrometry (HRMS) results of the solution which contains probe **HgP1** and Hg²⁺ showed an obvious peak at m/z = 353.0856, which should be assigned to the species compound **QCHO** (calculated 353.0926) (Fig. S8). The HRMS data further confirmed the above recognition mechanism shown in Scheme 2.

3.3. Biological imaging application experiment

In order to further explore the practical application value of probe **HgP1**, we used fluorescence inverted microscope to conduct cell imaging experiment in biological living cells (human esophageal cancer cell, Kyse30) to recognize exogenous Hg²⁺ [41–43]. First, we investigated the cytotoxicity of the probe by MTT assay. MTT results showed that the probe **HgP1** has weak cytotoxicity and could be used in living samples (Fig. 5). Then, the imaging experiment of recognizing exogenous Hg²⁺ by probe **HgP1** in living Kyse30 cells was carried out. As shown in Fig. 6,











Fig. 5. Viability of living cells treated with various concentrations of HgP1 $(3.0, 6.0, 8.0, 20, 50, 70 \,\mu\text{M})$ measured by MTT assay (24 h). The cell viability data were checked by three independent experiments.

the single probe HgP1 (10 μ M) showed almost no fluorescence after being incubated in living cells for 30 min (Fig. 6b). When Hg²⁺ (10 μ M) was added and incubated for another 30 min, the cells showed obvious green fluorescence (Fig. 6e). At the same time, the white light test results showed that the cells were in good condition during the above experiments (Fig. 6a and 6d). The results demonstrated that the probe HgP1 can be used in biological detection of Hg²⁺.

3.4. Direct detection of Hg^{2+} in real water samples

In order to further investigate the practical application value of probe HgP1, probe HgP1 was applied to detect the content of Hg²⁺ in



Fig. 6. The fluorescence imaging experiment of recognizing exogenous Hg^{2+} by **HgP1** in living cells. b) The images of **HgP1** (10 μ M) under green light in kyse30 cells; e) images of **HgP1** (10 μ M) and Hg²⁺ (10 μ M) in kyse30 cells under green light; a) and d) are the corresponding images of b) and e) under white light; c) is the merge images of a) and b), f) is the merge images of d) and e). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drinking water and tap water. The standard addition method of different concentrations of Hg^{2+} in drinking water and tap water was carried out. As shown in Table 1, by adding different concentrations of Hg^{2+} , the recovery results and recovery rates are satisfactory. The above experimental results further demonstrate the application value of probe HgP1 in practical samples.

Table 1

Recovery experiments (n = 4) of target Hg²⁺ in real water samples.

Sample number	Water	Added c/ (µmol·L ⁻¹)	Found c/ (µmol·L ⁻¹)	Recovery R/%
1	Tap water	3.6	$\textbf{3.49} \pm \textbf{0.25}$	96.9
2	Tap water	4	$\textbf{4.43} \pm \textbf{0.07}$	110.7
3	Drinking water	3.6	$\textbf{3.83} \pm \textbf{0.01}$	106.3
4	Drinking water	4	$\textbf{4.07} \pm \textbf{0.06}$	101.7

4. Conclusions

In conclusion, a new "turn-on" fluorescent probe which contains a dithioacetal group for the detection of Hg^{2+} was constructed by a simple one-step reaction. Based on the strong thiophilic affinity, probe **HgP1** displayed high selectivity and sensitivity to Hg^{2+} . The minimum detection limit of the probe is 8.2 nM. The results of biological imaging experiments showed that the probe can be applied in living cells.

CRediT authorship contribution statement

Zhiwei Ma: Writing - review & editing, Methodology. Di Zhang: Writing - review & editing, Data curation, Funding acquisition, Resources. Jie Guo: Investigation, Methodology, Formal analysis. Man Li: Data curation, Methodology. Tieliang Wang: Data curation, Software. Haiyan Yin: Software. Hongqi Wang: Data curation. Jihong Liu: Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.inoche.2021.108753.

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