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RAPID COMMUNICATION

A Disulfide-Linked Naphthalimide Dimer for Hg(II) Detection in Aqueous Solution

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Abstract A disulfide linked naphthalimide dimer probe was designed for mercury ion (Hg^{2+}) recognition in this work. The recognition was based on the strong affinity of mercury for sulfur. The experimental results revealed that the probe exhibited high selectivity and sensitivity toward Hg^{2+} in comparison to other metal ions *via* a turn-on and reversible response to Hg^{2+} in neutral buffer solution. More importantly, the probe demonstrated a linear response for Hg^{2+} over a concentration range from 0 to 150 μM with a detection limit of 0.38 μM, which is just the limit of the safe concentration for humans. Upon addition of 150 μM Hg^{2+}, the enhancement of fluorescence reached a maximum (~7-fold). The performances of the probe indicated that it could meet the selectivity requirements for biomedical and environmental application and also was sensitive enough to detect Hg^{2+} in environmental and biological samples.

Keywords Mercury ion · Fluorescent probe · Naphthalimide derivative · Turn-on · Reversible

Introduction

Mercury ion (Hg^{2+}) is one of the most hazardous and toxic heavy and transition metal ions (HTM) for the environment and human health. Mercury and its derivatives are widely distributed in air, water, and soil [1]. They can accumulate in the human or animal bodies through eating of contaminated organisms. Due to its inherent high toxicity, a wide variety of diseases are related with Hg^{2+} even in a low concentration such as prenatal brain damage, serious cognitive and motion disorders, and Minamata disease [2–5]. To ensure human health, the Environmental Protection Agency of the United States sets the upper limit of Hg^{2+} levels in drinking water as 2 ppb (10 nM) only [6]. Unfortunately, unlike some biological metal ions (such as Fe^{2+}, Mn^{2+} or Cu^{2+}), Hg^{2+} belongs to the so-called "silent ions" for that it does not have any intrinsic spectroscopic or magnetic signals because of its $5d^{10}$ $6s^0$ electronic configuration [7]. Therefore, these environmental and health problems as well as the intrinsic properties of Hg^{2+} have promoted the development of methods for the detection and the quantification of Hg^{2+}, especially under some circumstances where traditional detection techniques are not appropriate. Small-molecule fluorescent probes are a category for Hg^{2+} detection, they can selectively bind or react to Hg^{2+} and give out the fluorescent signal change for the sensing. These probes are well-suited not only for quick detection of Hg^{2+} in the field but also for in vivo studies in biological systems [8, 9]. In recent years, great efforts have been devoted for the designing of various small-molecule fluorescent probes specific for Hg^{2+} detection [10–23]. Depending on the spectral changes of the probe before and after impacting to Hg^{2+}, mercury fluorescent probes have been divided into "turn-on" (enhancement of fluorescence in the presence of Hg^{2+}) and "turn-off" probes (quenching of fluorescence by Hg^{2+}). However, the "turn-on" fluorescent probes for Hg^{2+} are particularly valuable because, like many other heavy metals, Hg^{2+} often causes fluorescence quenching via enhanced spin-orbit coupling associated with the heavy atom effect [24–27]. To date, most of the "turn-on" fluorescent probes for Hg^{2+} were based on the ring-opening of rhodamine and its derivatives mechanism [10, 28–34], which

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have some inherent limits, for example, the ring-opening process is somewhat dependent on the solvent system; the pH may also induce the ring-opening process of the rhodamine moiety; the Stokes shift of rhodamine is small, which poses potential difficulties for quantitative determination and bioimaging [35]; and some based on chemical reactions for the measurement of Hg^{2+} are irreversible [19, 36–38]. In addition, some macrocycles moieties were used as the receptor for Hg^{2+} [1, 7, 39–41], but the syntheses of these probes are complicated or low-yielding. Therefore, challenges still remain in designing fluorescent probes for Hg^{2+}, such as the high sensitivity, wide linear range, turn-on and reversible response, the large Stokes shift, simple procedure for synthesis, and improving practical application in environmental monitoring and biological systems.

In an effort to address these challenges, we have explored the possibility of developing a novel Hg^{2+} probe. Recently, we have designed a thiorhodamine-based chemdosimeter for Hg^{2+} recognition by virtue of the strong affinity of mercury for sulfur [42]. However, some unfavorable drawbacks still remain for practical monitoring such as the narrow linear range, the small Stokes shift and the irreversibility.

In our endeavour to develop a new Hg^{2+} probe to overcome these disadvantages, the naphthalimide moieties are found very attractive for construction of molecular probes because of their characteristics, such as the large Stokes shift, high fluorescence quantum yield, the modest excitation and emission wavelength, high photostability, high absorption coefficient, low cost and chemical reactions [5]. In all these merits, the properties of the large Stokes shift and high fluorescence quantum yield are especially in favor of the stability, dependability and sensitivity of the detection method. Recently, we have also designed some small molecular fluorescent probes with naphthalimide derivatives as the fluorophores [43, 44]. According to the soft and hard acid–base theory, the soft ligands (especially sulfur) as receptors can improve the selectivity toward Hg^{2+} [29, 45, 46]. Inspired by these works and analysis, we designed and synthesized the naphthalimide-based probe **2NASS**, a disulfide linked dimer, for Hg^{2+} recognition on the basis of the strong affinity of mercury for sulfur.

Experimental

Apparatus

Fluorescence measurements were taken on a Hitachi F-4600 fluorescence spectrometer with a 10 mm quartz cuvette. The excitation and emission slits were both set at 2.5 nm. All pH measurements were made with a Sartorius basic pH-meter PB-10. ^{1}H-NMR spectra were obtained on a Bruker AV-400 spectrometer with chemical shifts recorded in ppm (in DMSO-d_6, TMS as internal standard). Mass spectral analyses were carried out on a MALDI-TOF spectrometer. High-resolution mass data were measured with a Fourier transform ion cyclotron resonance mass spectrometer (APEXIV).

Reagents and Chemicals

All chemicals used in this work were of analytical-reagent grade, obtained from commercial suppliers and used without further purification. A stock solution of 0.01 M Hg^{2+} was prepared by dissolving 45.3 mg $Hg(ClO_4)_2$ in 10 mL double-distilled water. A stock solution of 1 mM **2NASS** was also prepared in DMF (*N,N*-dimethylformamide). A 1 M Tris–HCl buffer solution (pH 7.4) was employed. Double-distilled water was used throughout the experiment.

Synthesis of 2NASS

2NASS was prepared through an easy method as described in our previous report [43] (Scheme 1). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (*10^{-6}): 0.92(t, *J*=7.4 Hz, 6 H), 1.31–1.37(m, 4 H), 1.57–1.60(m, 4 H), 3.17(t, *J*=6.4 Hz, 4 H), 3.98(t, *J*=7.4 Hz, 4 H), 4.48(t, *J*=6.4 Hz, 4 H), 7.71(t, *J*=7.8 Hz, 2 H), 8.04(d, *J*=8.4 Hz, 2 H), 8.27(d, *J*=8.4 Hz, 2 H), 8.37(d, *J*=6.8 Hz, 2 H), 8.56(d, *J*=8.4 Hz, 2 H), 10.19(s, 2 H). MALDI-TOF calcd for $C_{38}H_{38}N_4NaO_8S_2$ $[M+Na]^+$ 765.2, found 765.2; $C_{38}H_{38}KN_4O_8S_2$ $[M+K]^+$ 781.2, found 781.2. HRMS (ESI positive) calcd for $C_{38}H_{39}N_4O_8S_2$ $[M+H]^+$ 743.22038, found 743.21947; $C_{38}H_{38}N_4NaO_8S_2$ $[M+Na]^+$ 765.20233, found 765.20083.

Procedure

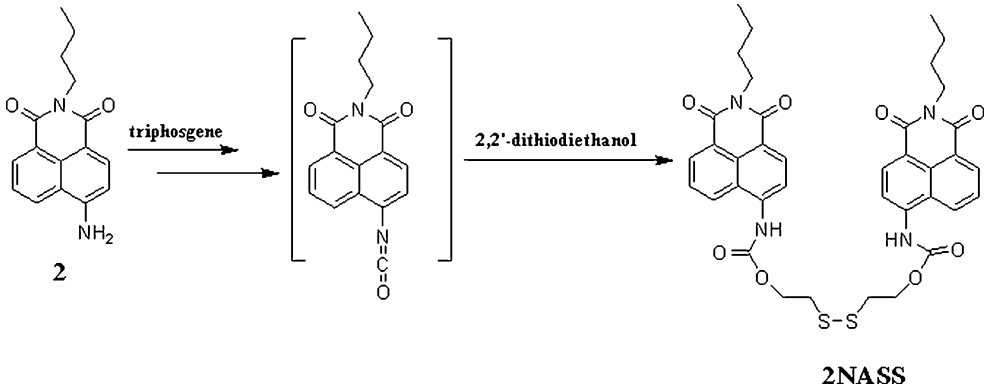
The fluorogenic samples were prepared in a 10-mL volumetric tube. Typically, to a test tube containing 5 mL of ethanol and 50 μL of 1 mM **2NASS**, 3 mL of double-distilled water was added. After 50 μL of 1 M Tris–HCl buffer solution (pH 7.4) was added, different concentration of Hg^{2+} was added and the mixture was diluted to 10.0 mL with water. The fluorescence intensity of the prepared solution was recorded at an emission wavelength of 468 nm with excitation wavelength set at 370 nm. At the same time, a solution containing no Hg^{2+} was prepared and measured under the same conditions as the control.

Results and Discussion

Measurement Media

Previous studies showed that the proper balance between hydrophilicity and lipophilicity of probes was favorable for both cell permeability and intracellular fluorescent imaging

Scheme 1 Synthesis of probe **2NASS**



[22, 35, 47–49]. Thus, the recognition of Hg^{2+} with **2NASS** was investigated under a mixture of ethanol and water (1:1, *v*/*v*) solution containing Tris–HCl buffer (5 mM, pH 7.4).

Spectral Characteristics

The fluorescence titration of Hg^{2+} was carried out with 5 μM **2NASS** solution in Tris–HCl (5 mM) buffer (ethanol/water = 1:1, *v*/*v*, pH 7.4). As illustrated in Fig. 1, with the increasing of Hg^{2+} concentration, the fluorescence intensity at 468 nm increased as well. Linear responses of **2NASS** as a function of Hg^{2+} concentration was observed ranging 0–150 μM (Fig. 2). The linear equation was found to be $y=10.271x+310.11$ ($R^2=0.9979$) where x represents the concentration of Hg^{2+} added and y as related fluorescence intensity measured at 468 nm. The detection limited was calculated to be 0.38 μM (3σ) [43], which is just the limit of the safe concentration for humans [48, 50].

Effect of pH

The effect of pH on the fluorescence response of **2NASS** was also investigated. The pH of the solution was adjusted by varying the amount of hydrochloric acid and sodium hydroxide while fixing the **2NASS** concentration at 5 μM. As shown in Fig. 3, experimental results showed that **2NASS** was stable in the pH range from 3.0 to 10.0, suggesting that it was actually pH-independent between that range and could work in real samples.

Selectivity Studies

Achieving high selectivity for the analyte of interest in a complex matrix containing potentially competing species is a challenging task in sensor development. The selectivity of **2NASS** to the various metal ions was investigated. In Fig. 4, **2NASS** showed extraordinary selectivity to Hg^{2+}. Upon addition of the same amount of the various metal ions, respectively, only Hg^{2+} induced a striking enhancement of fluorescence intensity (~7-fold). It revealed that **2NASS** could discriminate Hg^{2+} from other mental ions by fluoroscopy. To test practical applicability of **2NASS** for Hg^{2+}, competition experiments were also carried out. As illustrated in Fig. 5, **2NASS** showed almost unchanged responses to Hg^{2+} before and after addition of other interfering metal ions. Only Cu^{2+} and Fe^{3+} had minor

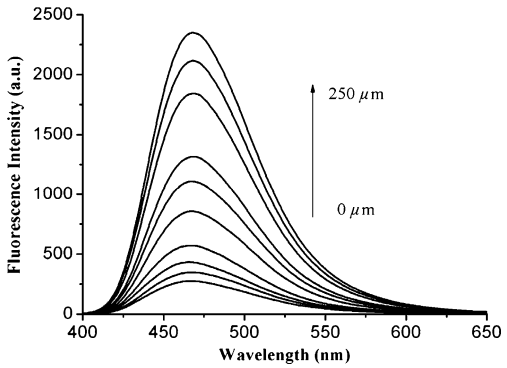


Fig. 1 Fluorescence responses of **2NASS** (5 μM) upon addition of Hg^{2+} (0, 5, 10, 25, 50, 75, 100, 150, 200, 250 μM) in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4). Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

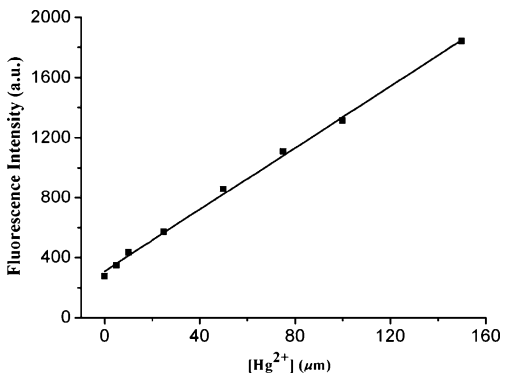


Fig. 2 Linear responses of **2NASS** to Hg^{2+} ranging from 0 to 150 μM at 468 nm in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4). Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

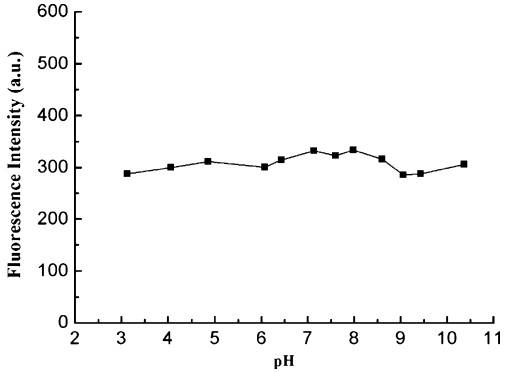


Fig. 3 Fluorescence responses of **2NASS** (5 μM) at 468 nm in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*) as a function of different pH values. Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

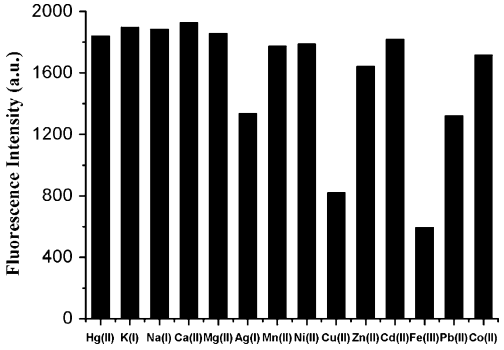


Fig. 5 Fluorescence responses of **2NASS** (5 μM) to Hg^{2+} (150 μM) in the presence of other metal ions (150 μM) at 468 nm in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4). Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

interference with the fluorescence response toward Hg^{2+}. All these selective results demonstrated that **2NASS** could meet the selective requirements for biomedical and environmental monitoring application.

Reversibility Studies

Reversibility of the probe may also be important for attributing a signal to Hg^{2+}, rather than some other factors, and for recycling of the probe [9]. We subsequently studied the chemical reversibility behavior of the metal binding of **2NASS** in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4). Because of the high stability constant of the EDTA-Hg^{2+} complex, it was anticipated that addition of EDTA will sequester Hg^{2+} of the metal complex, which released free **2NASS**. With this intention, adding of 1 equiv. of EDTA solution to the Hg^{2+} complex of **2NASS** results in an immediate return of the original metal free spectrum, which demonstrates the metal binding of **2NASS** is chemically reversible (see in Fig. 6).

Conclusions

In summary, the naphthalimide-based probe **2NASS**, a disulfide linked dimer, was designed for Hg^{2+} recognition. **2NASS** demonstrated remarkably selectivity toward Hg^{2+} in the presence of other metal ions. Most importantly, **2NASS** was capable for quantitative detection of Hg^{2+} *via* a turn-on and reversible fluorescent response with the linear range 0–150 μM and the detection limited at 0.38 μM. The fluorescent enhancement of **2NASS** was about 7-fold in the presence of Hg^{2+} (150 μM). All these selective and sensitive results indicate that **2NASS** could be used to determine Hg^{2+} in environmental and biological samples.

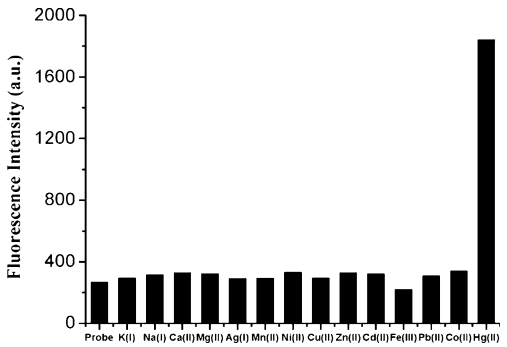


Fig. 4 Fluorescence responses of **2NASS** (5 μM) at 468 nm in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4) after the addition of 150 μM of various metal ions. Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

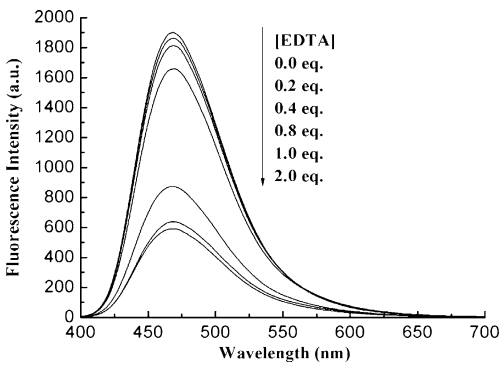


Fig. 6 Fluorescence responses of **2NASS**-Hg^{2+} complex in the presence of EDTA in Tris–HCl (5 mM) solution (ethanol/water = 1:1, *v*/*v*, pH 7.4). Excitation wavelength was set at 370 nm with excitation and emission slit width of 2.5 nm

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