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# *N*-(3-Imidazolyl)propyl dansylamide as a selective $Hg^{2+}$ sensor in aqueous media through electron transfer



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### Ashwani Kumar, Hong-Seok Kim\*

Department of Applied Chemistry, School of Applied Chemical Engineering, Kyungpook National University, Daegu 702-701, Republic of Korea

#### HIGHLIGHTS

- A highly Hg<sup>2+</sup> selective chemosensor **1** was synthesized and investigated.
- N-(3-Imidazolyl)propyl dansylamide acting as a selective Hg<sup>2+</sup> sensor in aqueous media.
- The sensor showed high selectivity for Hg<sup>+2</sup> even in the presence of other interfering metals (Ag<sup>+</sup>, Cu<sup>+2</sup>, Zn<sup>+2</sup>).
- The detecting mechanism of Hg<sup>2+</sup> was based on the electron transfer (ET).

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#### G R A P H I C A L A B S T R A C T



#### ABSTRACT

*N*-Imidazolylpropyl dansylamide **1** was synthesized for the sensing of metal ions and found to be selective and sensitive toward Hg<sup>2+</sup> ions in a PBS–EtOH (1:4, pH = 7.4) solution. The sensing ability of probe **1** was examined by UV–Vis, fluorescence, and <sup>1</sup>H NMR spectroscopy. The sensing of Hg<sup>2+</sup> exhibited a quenching of emission band at  $\lambda_{max} = 515$  nm of probe **1**, which was associated with quenching of green fluorescence emission under 365 nm illumination. Probe **1** showed a good association constant with Hg<sup>2+</sup> ( $K_a = 6.48 \times 10^4 \text{ M}^{-1}$ ) with a stoichiometry of 1:1 in PBS–EtOH (1:4, pH = 7.4) having the lowest detection limit of 1 µM for Hg<sup>2+</sup>; on the other hand, probe **2**, which has no imidazole motely, was not able to detect any metal ion. In the case of probe **1**, electrons on the imidazole nitrogen are available for electron transfer (ET), which was responsible for its green emission band that was quenched on addition of Hg<sup>2+</sup>; this clearly indicates that these electrons were used for the formation of a coordinate bond with Hg<sup>2+</sup> and that ET was switched off.

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#### Introduction

Metal ions play an essential role in about one third of the known enzymes; in particular, they can modify the electron flow in a substrate or enzyme, thus effectively controlling an enzyme-catalyzed reaction [1–5]. Without the appropriate metal ion, a biochemical reaction catalyzed by a particular metalloenzyme may proceed, if at all, very slowly. Some of the metal ions, such as mercury and lead,

are very toxic to organisms, including human [6–8]. The high affinity of mercury and its derivatives for thiol groups in proteins and enzymes causes dysfunction of cells, leading to health problems. Moreover, it can accumulate in plants, thereby reducing the rate of photosynthesis and transpiration, and in the human body, resulting in a variety of damaging health effects such as prenatal-brain damage, cognitive disorders, and immune system dysfunction, even at relatively low concentrations. Methyl-mercurial species are readily absorbed by the human gastrointestinal (GI) tract; they can cross the blood–brain barrier and target the central nervous system [9–15]. A noteworthy epidemic occurred in Iraq following

<sup>\*</sup> Corresponding author. Tel.: +82 53 9505588; fax: +82 53 9506594. *E-mail address:* kimhs@knu.ac.kr (H.-S. Kim).

the use of methyl mercury-tainted seed grain for bread. The United States Environmental Protection Agency (EPA) has mandated an upper limit of 2 ppb (10 nM) for  $Hg^{2+}$  in drinking water [16]. The health concerns over exposure to mercury have motivated the exploration of selective and efficient methods for the monitoring of mercury in biological and environmental samples. Fluorescent molecules appended with different types of architectures, such as thioether containing crown ethers/acetals [17-19], thioureas [20-23], podands [24-27], amines/amides [28-33], spirolactones [34-35], and heterocycle-based moieties [36-40], are used for Hg<sup>2+</sup> sensing. Most of these are chemodosimeters, i.e., irreversibly selective Hg<sup>2+</sup> sensors. Others are chemosensors, i.e., they can reversibly detect Hg2+, albeit non-selectively, showing some interference from either Cu<sup>2+</sup>/Ag<sup>+</sup> [41–45]. In continuation of our research efforts on metal ion sensing [46–52], in this study we focus on Hg<sup>2+</sup> sensing in aqueous media.

Dansyl, which is a good fluorescent tag used extensively in research, has a variety of binding sites for metal ion sensing [53–57]. Thus, we synthesized a dansyl-appended amide of 3-aminopropylimidazole/n-butylamine, i.e., probes **1** and **2**, respectively, for metal ion sensing in aqueous media. These probes were easily synthesized in a single step by the reaction of dansyl chloride with the corresponding amine, e.g., 3-aminopropylimidazole/n-butylamine in good yield.

#### **Results and discussion**

Probe **1** was easily synthesized by the reaction of dansyl chloride with 1(3-aminopropyl)-imidazole in dichloromethane in good yield (Scheme 1). The <sup>1</sup>H NMR spectrum of probe **1** in DMSO-d<sub>6</sub> showed three CH<sub>2</sub> propyl-chain groups: two as multiplet at  $\delta$ 1.67–1.74, 2.69–2.74, and one as a triplet at  $\delta$  3.86 for SO<sub>2</sub>NHCH<sub>2</sub>; one singlet at  $\delta$  2.82 for six protons of –N(CH<sub>3</sub>)<sub>2</sub>; and three imidazole protons as singlet at  $\delta$  6.88, 6.97, and 7.53 for the H-b, -c, and, -a protons, respectively. In the <sup>13</sup>C NMR spectrum of probe **1**, imidazole C-2 appears at  $\delta$  151.83. The high-resolution mass spectrum (HRMS) of probe **1** clearly showed a molecular ion peak for  $[M+H]^+$  at m/z = 359.1543 (see ESI). Similarly, probe **2** was synthesized in a single step from dansyl chloride and characterized by <sup>1</sup>H and <sup>13</sup>C NMR as well as HRMS.

The UV–Vis absorption spectrum of probe **1** (20  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) exhibited absorption maxima at  $\lambda_{max}$  = 338 nm. Upon addition of different metal ions, such as Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ga<sup>3+</sup>, In<sup>3+</sup>, and Hg<sup>2+</sup>, only Hg<sup>2+</sup> showed a significant red shift ( $\lambda_{max}$  = 370 nm); the other metal ions did not show any significant changes in the UV–Vis spectra of probe **1** (Fig. SI 1).

Excitation of probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) at 338 nm ( $\lambda_{max}$  in the UV–Vis spectra of probe **1**) resulted in fluorescence emission maxima at 515 nm with high quantum yield ( $\Phi$  = 0.31). Upon addition of different metal ions, such as Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ga<sup>3+</sup>, In<sup>3+</sup>, and Hg<sup>2+</sup>, only Hg<sup>2+</sup> caused quenching of the green emission band at  $\lambda_{max}$  = 515 nm; other metal ions did not show any significant changes in the fluorescence emission band of probe **1** (Figs. 1 and SI 2).

Upon gradual aliquot additions of Hg(ClO<sub>4</sub>)<sub>2</sub> to probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4), the emission intensity at 515 nm slowly decreased until saturation. The fitting of these fluorescence titration data shows the formation of a 1:1 complex with a good association constant ( $K_a = 6.48 \times 10^4 \text{ M}^{-1}$ ) (Fig. 2). Probe **1** can detect Hg<sup>2+</sup> in the range of 1–11  $\mu$ M: that was determined from the linear relationship between fluorescence emission intensity at  $\lambda_{max} = 515 \text{ nm}$  i.e.,  $I_{515} \text{ vs}$  [Hg<sup>2+</sup>] having R = 0.9969 on titration of probe **1** with Hg<sup>2+</sup> (Fig. SI 3). The formation of the 1:1 complex of probe **1** with Hg<sup>2+</sup> ion was also observed from Job's plot (Fig. 3).

In order to check their interference, 10 equiv of other metal ions were added to the solution of  $1 + [Hg^{2+}]$  (1:1)-complex; it was found that probe 1 could detect  $Hg^{2+}$  ions even in the presence of other metal ions (Fig. 4).

In the case of probe **2** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4), excitation at 338 nm ( $\lambda_{max}$  in the UV–Vis spectra of probe **2**) resulted in



**Scheme 1.** Synthesis of probes **1** and **2**.



**Fig. 1.** Fluorescence relative intensity of probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) on addition of 10 equiv of different metal ions ( $\lambda_{ex}$  = 338 nm,  $\lambda_{em}$  = 515 nm); also in the figure, visual fluorescence color change of probe **1** (3  $\mu$ M) with Hg<sup>2+</sup> (10 equiv) under illumination at 365 nm is shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Fluorescence titration of probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) with Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{ex}$  = 338 nm. The inset shows the curve fitting; points show the experimental values and line shows the curve fit at *I*<sub>515</sub> vs [Hg<sup>2+</sup>], respectively.



**Fig. 3.** Job's plot for the complex of probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) with Hg(ClO<sub>4</sub>)<sub>2</sub>,  $\lambda_{ex}$  = 338 nm,  $\lambda_{em}$  = 515 nm.

fluorescence emission maxima at 515 nm. Upon addition of different metal ions, such as Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Ga<sup>3+</sup>, In<sup>3+</sup>, and Hg<sup>2+</sup>, no significant changes in the fluorescence emission intensity of probe **2** were observed (Fig. 5).

The fluorescence study of probe **2** clearly showed the relevance of the imidazole ring in probe **1**, which provides the appropriate binding site for the electron transfer (ET) for the detection of



**Fig. 5.** Fluorescence study of probe **2** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) upon addition of different metal ions;  $\lambda_{ex}$  = 338 nm,  $\lambda_{em}$  = 515 nm.

Hg<sup>2+</sup> ions. In order to further investigate the mode of complexation and binding nature of Hg<sup>2+</sup> ions with probe **1**, the <sup>1</sup>H NMR titration of probe **1** with Hg<sup>2</sup> ions in DMSO-d<sub>6</sub> was carried out (Figs. 6 and SI 3); DMSO-d<sub>6</sub> was used because precipitation was observed in CD<sub>3</sub>OD.

The addition of 1 equiv of  $Hg(ClO_4)_2$  to the solution of probe 1 in DMSO-d<sub>6</sub> led to a significant downfield shift of the imidazole protons labeled as  $H_a$ ,  $H_b$ , and  $H_c$  from  $\delta$  7.530 to 8.365,  $\delta$  6.880 to 7.340 and  $\delta$  6.966 to 7.616 respectively; while H<sub>g</sub> of the dansyl unit also shifted downfield from  $\delta$  8.458 to 8.479; whereas protons H<sub>1</sub> shifted upfield from  $\delta$  8.283 to 8.266. Because of the active nature of sulfonamide N-H signal (H<sub>n</sub>), however, disappeared, probably due to the deprotonation induced by Hg<sup>2+</sup>, which was also supported by the significant upfield shift of the H<sub>1</sub> proton. Similarly, the aliphatic signals of the propyl-chain i.e., protons d, e and f are shifted downfield from  $\delta$  2.710 to 2.745,  $\delta$  1.710 to 1.870 and  $\delta$  3.860 to 4.210 respectively; this effect was more pronounced in the case of CH<sub>2</sub> (f-protons). No changes were observed in the methyl signals (m-protons) of N,N-dimethyl (Fig. SI 4). Upon a further addition of 1 equiv of  $Hg(ClO_4)_2$ , no further shift of the proton signals was observed, indicating the formation of the 1:1 complex between probe 1 and Hg<sup>2+</sup>. In this case, the electrons on the nitrogen of the imidazole ring were available for electron transfer



**Fig. 4.** Interference study of probe **1** (3  $\mu$ M, PBS–EtOH (1:4), pH = 7.4) upon addition of different metal ions to the solution of **1** + [Hg<sup>2+</sup>](1:1)-complex,  $\lambda_{ex}$  = 338 nm,  $\lambda_{em}$  = 515 nm.



Fig. 6. (i) Partial <sup>1</sup>H NMR spectra of probe 1; (ii) upon addition of 1 equiv of Hg(ClO<sub>4</sub>)<sub>2</sub>; (iii) 2 equiv of Hg(ClO<sub>4</sub>)<sub>2</sub> in DMSO-d<sub>6</sub>.



Fig. 7. The proposed scheme for complexation of probe 1 with Hg<sup>2+</sup> ion.

(ET), which was responsible for the green emission band at  $\lambda_{max} = 515$  nm that was quenched upon addition of Hg<sup>2+</sup>; this clearly indicates that these electrons were used for the formation of the coordinate bond with Hg<sup>2+</sup> and that ET was switched off. Thus, based on the results obtained from fluorescence experiments, <sup>1</sup>H NMR titrations, and job's plot, we proposed a binding model of Hg<sup>2+</sup> ion detection by probe **1** as shown in Fig. 7.

#### Conclusions

In this work, probe **1** was found to act as a good sensor for  $Hg^{2+}$  ions in aqueous solution. The probe enabled the visual detection of  $Hg^{2+}$  through a color change under 365 nm illumination accompanied with the quenching of green emission band at  $\lambda_{max} = 515$  nm through ET having lowest detection limit of 1  $\mu$ M for  $Hg^{2+}$ .

#### **Experimental section**

#### General

The melting points were determined using a Thomas-Hoover capillary melting-point apparatus and were uncorrected. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker AM-400 spectrometer using Me<sub>4</sub>Si as the internal standard. The HR-FAB mass was determined at the KBSI Daegu branch. The UV–Vis absorption spectra were obtained with a Shimadzu UV-1650PC spectrophotometer. The fluorescence spectra were measured on a Shimadzu RF-5301 fluorescence spectrometer equipped with a xenon discharge lamp using 1 cm quartz cells with slit width 5 nm. All the

measurements were performed at 298 K. Analytical grade ethanol was purchased from Merck. All other materials for synthesis were purchased from Aldrich Chemical Co. and used as received. The quantum yield ( $\Phi$ ) was calculated using the procedure reported in the literature [58].

#### Synthesis of probes 1 and 2

#### Probe 1

To a solution of dansyl chloride (134 mg, 0.5 mmol) in dichloromethane (10 mL), which was stirred at 0-10 °C under argon atmosphere, 1(3-aminopropyl)imidazole (125 mg, 1.0 mmol) was added dropwise. Triethylamine (202 mg, 2.0 mmol) was then added for neutralization of the generated acid; the reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the reaction mixture was concentrated and purified by silica gel column chromatography with CH<sub>2</sub>Cl<sub>2</sub>: CH<sub>3</sub>OH (9:1) as eluent ( $R_f = 0.6$ ) to obtain yellow solid **1** (90 mg, 50%). mp. 72 °C (CH<sub>2</sub>Cl<sub>2</sub>-hexane); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 1.67–1.74 (m, 2H, CH<sub>2</sub>), 2.69–2.74 (m, 2H, CH<sub>2</sub>), 2.82 (s, 6H, 2 x CH<sub>3</sub>), 3.86  $(t, I = 6.8 \text{ Hz}, 2H, CH_2), 6.88 (s, 1H, ImH_b), 6.97 (s, 1H, ImH_c), 7.26$ (d, J = 6.8 Hz, 1H, ArH), 7.53 (s, 1H, ImH<sub>a</sub>), 7.61 (t, J = 8.0 Hz, 2H, 2 x ArH), 8.02 (t, / = 6.0 Hz, 1H, SO<sub>2</sub>NH), 8.07 (d, / = 8.0 Hz, 1H, ArH), 8.28 (d, J = 8.8 Hz, 1H, ArH), 8.46 (d, J = 8.8 Hz, 1H, ArH); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 31.12 (CH<sub>2</sub>), 39.80 (CH<sub>2</sub>), 43.42 (CH<sub>2</sub>), 45.43 (2 x CH<sub>3</sub>), 115.55, 119.32, 119.42, 124.01, 128.30, 128.68, 128.81, 129.38, 129.45, 129.90, 136.12, 137.40, 151.83; HR-FAB mass calcd for:  $C_{18}H_{23}N_4O_2S (M + H)^+$ : 359.1542; Found: *m*/*z* 359.1543.

#### Probe 2

The procedure for the preparation of probe **2** is similar to that of probe **1**; dansyl chloride (134 mg, 0.5 mmol), *n*-butylamine (87 mg, 1.0 mmol), and triethylamine (202 mg, 2.0 mmol) were used. The crude product was purified by silica gel column chromatography with CH<sub>2</sub>Cl<sub>2</sub> as eluent ( $R_f$  = 0.8) to obtain a light-green thick liquid **2** (135 mg, 88%). liquid at room temperature; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  0.74 (t, *J* = 7.2 Hz, 3H, CH<sub>3</sub>), 1.14–1.23 (m, 2H, CH<sub>2</sub>), 1.32–1.39 (m, 2H, CH<sub>2</sub>), 2.87 (m, 2H, CH<sub>2</sub>), 2.92 (s, 6H, 2 x CH<sub>3</sub>), 4.70 (bs, 1H, SO<sub>2</sub>NH), 7.23 (d, *J* = 7.2 Hz, 1H, ArH), 7.52–7.59 (m, 2H, 2 × ArH), 8.25 (d, *J* = 7.6 Hz, 1H, ArH), 8.33 (d, *J* = 8.4 Hz, 1H, ArH), 8.59 (d, *J* = 8.4 Hz, 1H, ArH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  14.03, 20.08, 30.10, 34.40, 45.91, 115.73, 120.60, 123.73, 128.23, 130.16, 130.29, 130.49, 130.71, 134.87, 151.53; HR-FAB mass calcd for: C<sub>16</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>S (M)<sup>+</sup>: 306.1402; Found: *m/z* 306.1404.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.03.091.

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