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## COMMUNICATION

## A BODIPY-based fluorescent chemodosimeter for Cu(II) driven by an oxidative dehydrogenation mechanism<sup>†</sup>

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A boradiazaindacene (BODIPY) derivative containing a simple NO bidentate ligand shows a  $Cu^{2+}$ -selective fluorescence in aqueous media. This is promoted *via* a coordination of  $Cu^{2+}$  followed by oxidative dehydrogenation of an amine moiety, leading to a formation of a fluorescent  $Cu^+$ -Schiff base complex.

The design of fluorescent probes for selective detection of  $Cu^{2+}$  has attracted much attention because  $Cu^{2+}$  is a significant environment pollutant and yet an essential trace element in biological systems.<sup>1</sup> A variety of  $Cu^{2+}$  probes have been proposed; however, most of the early reported probes show fluorescence quenching response due to the paramagnetic nature of  $Cu^{2+}$  and lack sufficient sensitivity.<sup>2</sup>  $Cu^{2+}$  probes showing a fluorescence enhancement response have also been proposed; however, many of these show poor binding selectivity to  $Cu^{2+}$  over other cations such as Fe<sup>3+</sup> and Hg<sup>2+</sup> and lack sufficient selectivity.<sup>3</sup>

Recently, reaction-based Cu<sup>2+</sup> probes that are called chemodosimeters have attracted a great deal of attention,4-6 as an alternative to the classical chelation-based probes. In most cases, nonemissive chemodosimeter molecules are converted to the emissive ones *via* irreversible chemical reactions promoted by  $Cu^{2+}$ . A large number of fluorescent Cu<sup>2+</sup> chemodosimeters have been proposed based on several Cu2+-promoted reactions such as hydrolysis of lactone,<sup>4a</sup> esters,<sup>4b-e</sup> hydrazone,<sup>4f</sup> lactams,<sup>4g,h</sup> and amide;<sup>4i</sup> and oxidation of dihydrorosamine,<sup>5a</sup> phenothiazine,<sup>5b</sup> catechol,<sup>5c</sup> phenol,<sup>5d</sup> and DNA.<sup>5e</sup> A few chemodosimeters employ other reactions.<sup>6</sup> Many of these, however, suffer from several issues. They (i) act only in pure organic solvents or solutions with very small amounts of water (<20%);<sup>4di,5b-d6</sup> (ii) require specific reaction conditions such as high temperature,<sup>4a-c,6c</sup> acidic<sup>4f</sup> or basic pH;<sup>4a</sup> and, (iii) show low Cu<sup>2+</sup> selectivity in the presence of other metal cations.<sup>4e,g-i,5e,6a,c</sup> To the best of our knowledge, there is only one report of the Cu<sup>2+</sup> chemodosimeter which addresses these issues.<sup>5a</sup> The design of chemodosimeters based on a new  $Cu^{2+}$ -selective reaction is thus the current focus of attention.

Oxidative dehydrogenation of amine to imine is a well known reaction in biochemistry, promoted by coordination



Scheme 1 Cu<sup>2+</sup>-promoted dehydrogenation model for amine.<sup>8,9</sup>

of a transition metal cation such as  $Cu^{2+,7}$  As shown in Scheme 1, a tetrahedral coordination of amine-containing ligands with  $Cu^{2+}$  gives rise to the corresponding imine complexes in the presence of the molecular oxygen at room temperature,<sup>8,9</sup> where the reaction sometimes involves reduction of  $Cu^{2+}$  center to  $Cu^{+,9}$  Although the detailed mechanism is still unclear, cooperative oxidation by  $Cu^{2+}$  and  $O_2$  is commonly thought to be involved in the reaction.

Herein, we report that the  $Cu^{2+}$ -promoted dehydrogenation of amine allows selective chemodosimetric detection of  $Cu^{2+}$  in aqueous media with neutral pH at room temperature. We employ a ligand  $1^{10}$  (Fig. 1), consisting of a BODIPY dye<sup>11</sup> as a fluorescent signaling unit and a simple NO bidentate ligand as a coordination site for  $Cu^{2+}$ .<sup>8</sup> The ligand 1 is nonfluorescent, but  $Cu^{2+}$  addition shows a strong fluorescence enhancement. This is promoted *via* a coordination of  $Cu^{2+}$  with 1 followed by dehydrogenation of amine moieties, leading to a formation of a fluorescent  $Cu^+$ –Schiff base complex.

The ligand **1** was obtained according to the procedure described previously.<sup>10</sup> Compounds **2–4** (Fig. 1) were synthesized as reference materials, which were obtained with > 76% yields (see ESI†) and fully characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and MS analysis (Fig. S1–S9, ESI†).

Fig. 2a shows the fluorescence spectra ( $\lambda_{ex} = 510 \text{ nm}$ ) of 1 (5  $\mu$ M) measured in a buffered MeCN/water mixture (1/1 v/v; HEPES 100 mM; pH 7.0) with 20 equiv. of the respective metal cation in an aerated condition. Without cation, 1 shows



Fig. 1 Chemodosimeter 1 and reference compounds 2-4.

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**Fig. 2** (a) Fluorescence spectra ( $\lambda_{ex} = 510 \text{ nm}$ ) of **1** (5 µM) measured in a MeCN/water mixture (1/1 v/v; HEPES 100 mM; pH 7.0) with 20 equiv. of respective metal cation in an aerated condition. The spectra were measured after stirring the solution with cation for 20 min. (b) The ratio of fluorescence intensity (FI/FI<sub>0</sub>) of **1**. The absorption spectra are summarized in Fig. S10, ESI.†

almost no fluorescence, where the emission quantum yield ( $\Phi_{\rm F}$ ) is 0.002. This is because, as usually observed,  $^{1b}$  the electron transfer from the amine nitrogen to the excited state BODIPY moiety quenches the emission. Addition of Cu<sup>2+</sup>, however, creates a strong fluorescence at 520–680 nm ( $\Phi_{\rm F} = 0.095$ ). As shown in Fig. 2b, the fluorescence enhancement upon Cu<sup>2+</sup> addition is more than 256-fold. In contrast, other metal cations scarcely show fluorescence enhancement ( $\Phi_{\rm F}$  <0.005). It is noted that the fluorescence response of 1 to  $Cu^{2+}$  is unaffected by other metal cations (Fig. S11, ESI<sup>†</sup>), indicating that 1 detects  $Cu^{2+}$  selectively even in the presence of other cations. The Cu<sup>2+</sup>-induced fluorescence enhancement of 1 occurs at pH 5-9 (Fig. S12, ESI $\dagger$ ), suggesting that 1 enables Cu<sup>2+</sup> detection at a physiological pH range. In addition, counter anions of Cu<sup>2+</sup> do not affect the response of 1 (Fig. S13, ESI<sup>+</sup>). Furthermore, the fluorescence emission obtained upon Cu<sup>2+</sup> addition is stable; the intensity is kept for at least 30 days even left in an aerated condition at room temperature.

Fig. 3 shows the result of fluorescence titration of **1**. The  $Cu^{2+}$  addition leads to an increase in the 596 nm fluorescence, and the increase is saturated with 20 equiv. of  $Cu^{2+}$ . As shown in the inset, a linear relationship is observed between the



**Fig. 3** Fluorescence titration ( $\lambda_{ex} = 510 \text{ nm}$ ) of **1** (5  $\mu$ M) with Cu<sup>2+</sup> (0–30 equiv.) in a MeCN/water mixture (1/1 v/v; HEPES 100 mM; pH 7.0). Each spectrum was obtained after stirring the solution with Cu<sup>2+</sup> for 20 min. (inset) Change in intensity at 596 nm.



**Fig. 4** Absorption titration of **1** (5  $\mu$ M) with Cu<sup>2+</sup> (0–30 equiv.) in a MeCN/water mixture (1/1 v/v; HEPES 100 mM; pH 7.0).

intensity and Cu<sup>2+</sup> amount at the range of 0.05–4 equiv., which corresponds to 0.25–20  $\mu$ M Cu<sup>2+</sup>. The detection limit, 0.25  $\mu$ M, is below the maximum permissive level of Cu<sup>2+</sup> in drinking water (~20  $\mu$ M) set by the U.S. Environmental Protection Agency. The fluorescence increase of 1 after addition of Cu<sup>2+</sup> is saturated within 20 min (Fig. S14, ESI†), suggesting that 20 min assay time is enough for analysis.

Fig. 4 shows the result of absorption titration of **1**. Without a cation, **1** shows an absorption band at 605 nm, assigned to the BODIPY moiety.<sup>11</sup> The Cu<sup>2+</sup> addition leads to a decrease in this band with a blue shift (584 nm).<sup>12</sup> The spectral change almost stops upon addition of 20 equiv. of Cu<sup>2+</sup>, which is similar to the fluorescence titration result (Fig. 3). Adding excess amount of EDTA to the solution does not show further spectral change (Fig. S16, ESI†), indicating that **1** reacts with Cu<sup>2+</sup> irreversibly.

The Cu<sup>2+</sup>-induced emission enhancement is due to the formation of a 1–Cu<sup>+</sup> 2 : 1 complex containing two imine moieties (Scheme 2). This is promoted *via* a coordination of Cu<sup>2+</sup> with two 1 molecules followed by dehydrogenation by Cu<sup>2+</sup> and O<sub>2</sub>. The reaction of 1 with Cu<sup>2+</sup> in a 2 : 1 stoichiometry is confirmed by Job's plot (Fig. S18, ESI†); the emission intensity shows a maximum at X = 0.33 (=[Cu<sup>2+</sup>]/([Cu<sup>2+</sup>] + [1]). The solution of 1 treated with Cu<sup>2+</sup> was extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with water, and concentrated by evaporation. Fig. 5 shows the IR spectra of 1 and the obtained 1–Cu complex. 1 shows two distinctive bands assigned to the O–H and N–H stretching vibration at *ca*. 3450 cm<sup>-1</sup>. In contrast, the 1–=Cu complex does not show these bands, but shows a new band at 1618 cm<sup>-1</sup>, assigned to the imine (C=N) moiety. This band is also observed for a



Scheme 2 Proposed mechanism for the  $Cu^{2+}$ -promoted fluorescence enhancement of 1. The optimized geometry of the 1– $Cu^+$  2 : 1 complex (right) is shown in Fig. S17, ESI.†



Fig. 5 Partial IR spectra of 1, 2, and 1-Cu complex.

reference compound, **2**, containing an imine moiety (Fig. 1). These suggests that the **1**–Cu complex involves two imine moieties.<sup>13</sup> As shown in Fig. S20 (ESI<sup>†</sup>), ESR analysis of the complex shows no signal for Cu<sup>2+</sup>, indicating that the complex involves Cu<sup>+</sup>. These findings suggest that, as shown in Scheme 2 and observed for related amine-containing ligands,<sup>9</sup> the reaction of Cu<sup>2+</sup> with two **1** molecules produces a Cu<sup>+</sup>–Schiff base complex *via* an oxidative dehydrogenation of two amine moieties by Cu<sup>2+</sup> and O<sub>2</sub>.<sup>14</sup> The dehydrogenation of amine moieties of **1** suppresses the electron transfer from the nitrogen atoms to the excited state BODIPY moiety<sup>1b</sup> and results in emission enhancement (Fig. 2).

The reference compound 2 with an imine moiety (Fig. 1) shows a strong emission ( $\Phi_{\rm F} = 0.19$ ). Addition of Cu<sup>+</sup> to 2, however, shows almost no spectral change (Fig. S22, ESI†). This is because 2 scarcely coordinates with Cu<sup>+</sup> due to the low affinity to the phenolic –OH group.<sup>15</sup> In contrast, addition of Cu<sup>2+</sup> to 2 strongly quenches the emission. This is probably due to the paramagnetic nature of Cu<sup>2+</sup>.<sup>16</sup> Other reference compounds 3 and 4 that do not contain –OH groups (Fig. 1) show very weak emission enhancement upon Cu<sup>2+</sup> addition of Cu<sup>2+</sup> with –OH is crucial for amine dehydrogenation. These data also support the proposed mechanism (Scheme 2).

It is noted that the  $Cu^{2+}$ -promoted emission enhancement of 1 depends strongly on solvents. Addition of  $Cu^{2+}$  to 1 in water (pH 7) containing DMSO, THF, acetone, MeOH, DMF, or dioxane (50%) shows almost no emission enhancement. This is probably because  $Cu^+$  species is unstable in these solvents, although MeCN stabilizes  $Cu^+$  species.<sup>17</sup> In addition, as shown in Fig. S23 (ESI†), the 596 nm fluorescence does not appear in pure MeCN, but an increase in the water content leads to an enhancement. This is probably because vater catalyzes the  $Cu^{2+}$ -amine redox process. Although the detailed role of water cannot be identified at this stage, similar water effect is observed in the dehydrogenation of an amine ligand in the Fe<sup>3+</sup> complex system.<sup>18</sup> These findings suggest that both MeCN and water are necessary for the  $Cu^{2+}$ -induced fluorescence enhancement of 1.

In summary, we found that a BODIPY derivative, 1, containing a simple NO bidentate ligand behaves as a fluorescent chemodosimeter for  $Cu^{2+}$ . Although the detailed analysis for the dehydrogenation mechanism still remains to be clarified, the simple ligand design may contribute to the design of the more efficient and useful chemodosimeter for  $Cu^{2+}$ . This work was supported by the Grant-in-Aid for Scientific Research (No. 21760619) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT).

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