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# Development of a Fluorescent Molecular Probe for Analysis of Zinc Ion in Aquatic Samples

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## ABSTRACT

A novel asymmetric 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) fluoroionophore with terpyridine (BDP-TPY) is described. BDP-TPY shows a high affinity toward $Zn^{2+}$ and upon complexation, the fluorescence spectrum red-shifts from 539 nm to 567 nm. $Zn^{2+}$ concentration was successfully determined by ratiometric measurement. The dynamic range of $Zn^{2+}$ was $1.7 \times 10^{-8} - 1.0 \times 10^{-5}$ M. The spectral response could be very useful in the development of a novel ratiometric fluoroionophore for analyzing $Zn^{2+}$ in environmental samples.

## 1. INTRODUCTION

The conservation and protection of the natural environment from trace contaminants is becoming increasingly important (Yantasee 2007), and in particular, heavy metals pose severe risks to human health and to the environment because of their toxicity (Kawata 2007). For instance, Zinc (Zn) is also one of the major heavy-metal pollutants because of its widespread industrial applications in chemical and alloyed products, fabricated metal products, and paper products (Naito 2010). Zn compounds have hazardous effects not only on humans but also on plants and fish (Clearwater 2002). Currently, the most common analytical methods for heavy metals are atomic absorption spectrometry (AAS) (Adeloju 2009) and inductively coupled plasma (ICP) spectroscopy (Lafleur 2008). Although precise, these analytical instruments are of high cost and often require complex sample preparation. In addition, they are not applicable to continuous on-site monitoring. Therefore, a low-cost and simple method is needed for determining levels of heavy-metal ions, which is appropriate for monitoring industrial and environmental samples on site.

Fluorescence spectroscopy is a desirable method for quantifying heavy-metal ions because of its high sensitivity, operational simplicity, and versatile instrumentation (Lakowicz 2006). The design and development of novel fluoroionophores remains an active area of research in analytical chemistry (Shao 2005), and a numerous fluoroionophores for heavy-metal ions have been reported (Khan 2009). In terms of sensitivity, the fluoroionophores that exhibit ratiometric spectral changes induced by complexation with heavy-metal ions are more favorable than those exhibiting only fluorescence enhancement (“turn-on”) or fluorescence quenching (“turn-off”). Ratiometric fluoroionophores provide more detailed information about the analyte in a sample, as well as allowing reliable measurements of the analyte concentration, as the ratio of the fluorescence intensities at two wavelengths is independent of fluctuations of the source light intensity and sensitivity of the instrument (Valeur 2002). However, the development of ratiometric fluoroionophores is challenging.

Our concept for the ratiometric measurement of heavy-metal ions is based on the synthesis of an asymmetric 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) fluorophore. BODIPY fluorophores possess many valuable characteristics, such as sharp and intense absorption and fluorescence bands, high fluorescence quantum yields, high molar absorption coefficients, and good photo-chemical stability (Karolin 1994). Furthermore, since BODIPY fluorophores are amenable to structural modification, controlling the substituent pattern allows for changes in the wavelength of the absorption and fluorescence spectrum (Loudet 2007). Many asymmetric BODIPYs for metal ions have been reported (Yamada 2005), usually containing an amino group as a metal-ion receptor at the 3 position of the BODIPY core. However, there are no studies on asymmetric BODIPY, which has a pyridyl ion receptor at the 3 position. In this study, we present di(2-picolyl)amine substituted BODIPY (BDP-DPA) as a fluoroionophore for heavy-metal ions. $Zn^{2+}$ concentration was successfully determined by ratiometric measurement in an aqueous solvent system.

## 2. EXPERIMENTAL SECTION

### *2.1. Synthetic Procedure*

BDP-TPY was synthesized according to the previous report (Hafuka submitted).

### *2.2. Spectroscopic Measurements*

The spectroscopic measurements were carried out in an aqueous acetonitrile solution ($CH_3CN/H_2O=9/1$, v/v). Stock solutions of BDP-TPY were prepared by dissolving BDP-TPY in analytical grade acetonitrile. Stock solutions of metal ions were prepared by dissolving appropriate amounts of analytical grade perchlorate salts in a Tris-HCl buffer (0.01 M). Mill-Q water (18.25 MΩ cm) was used to prepare all aqueous solutions. Quartz cells (cross section of 1 cm × 1 cm) were used for fluorescence and absorption measurements. Fluorescence and absorption spectra were obtained on a JASCO FP-6600 spectrofluorometer and a JASCO V-630 spectrophotometer, respectively. The excitation and fluorescence slit widths were 5.0 and 6.0 nm, respectively.

Appropriate aliquots of the BDP-TPY stock solutions were dropped into 10-mL volumetric flasks, and then different kinds of metal-ion stock solutions were added to each flask. The solutions were diluted with Tris-HCl buffer and acetonitrile.

Different concentrations of metal-ion stock solutions were prepared in 10-mL volumetric flasks. Test solutions were prepared by adding a specific amount of the BDP-TPY stock solution and a moderate amount of the metal-ion stock solution into 10-mL volumetric flasks and then diluting with Tris-HCl buffer and acetonitorile. A detection limit (LOD, 3σ/slope) and a quantification limit (LOQ, 10σ/slope) for each metal ion were determined based on the standard deviation (σ) of 11 blank solutions (Shao 2010).

Appropriate aliquots of BDP-TPY stock solutions were dropped into 10-mL volumetric flasks, and then different kinds of the metal-ion stock solution were added to each flask. Following that, specific amounts of $Zn^{2+}$ stock solution were added to these flasks. Finally, the solutions were diluted with Tris-HCl buffer and acetonitrile.

## 3. RESULTS AND DISCUSSION

Fig. 1 shows the fluorescence spectra of BDP-TPY in the aqueous acetonitrile solution with and without the respective metal ions (50 eq.). Without the ions, BDP-TPY showed a greenish-yellow fluorescence at 539 nm and a shoulder around 580 nm, which is typical of BODIPY fluorophores (Karolin 1994). The spectra of BDP-TPY were shifted to longer wavelengths upon addition of $Zn^{2+}$, $Cd^{2+}$, and $Hg^{2+}$. The extent of the spectral shift depends on the ion species. Complexes of BDP-TPY with $Cd^{2+}$ and $Hg^{2+}$ showed red-shifted fluorescence bands at 563 nm and 561 nm, respectively, while $Zn^{2+}$ induced the largest bathochromic shift (from 539 nm to 567 nm) of the fluorescence band. Goze et al. (2003) reported that BODIPY fluoroionophores functionalized with terpyridine at the meso position showed fluorescence quenching toward $Zn^{2+}$. To the best of our knowledge, this is the first example of asymmetric BODIPY with a pyridyl receptor at the 3 position showing red-shifted fluorescence by complexation with metal ions. It is known that when electron-withdrawing groups (e.g., pyridyl group) are part of the fluorophore π-system and involved in ion binding, the excited state is more stabilized than the ground state upon complexation with metal ions, and therefore the energy gap is reduced (Coskun 2007). In BDP-TPY, the terpyridine moiety could interact with metal ions in this way, resulting in the observed bathochromic shift of the fluorescence spectra (Fig. 1). The red shift enables a ratiometric fluorescence measurement of the metal ions.

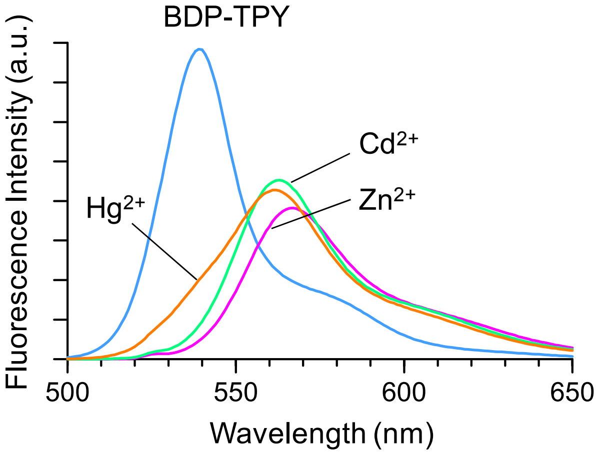

Fig. 1 Fluorescence spectra of BDP-TPY in the absence and presence of different metal ions (50 μM) in aqueous acetonitrile solution with excitation at 525 nm. The concentration of BDP-TPY was 1 μM.

Fig. 2 shows the $Zn^{2+}$-concentration-dependent fluorescence spectra of BDP-TPY in the aqueous acetonitrile solution. Fluorescence spectra of BDP-TPY upon titration with $Zn^{2+}$ displayed a bathochromic fluorescence shift from 539 nm to 567 nm and a distinct ratiometric change with a clear isoemission point at 551 nm. The spectral change was almost terminated by the addition of 10 eq. of $Zn^{2+}$. The red shift allows ratiometric measurement. Since fluorescence intensity at 567 nm (F567) increased while F539 simultaneously decreased with increasing concentration of $Zn^{2+}$, the ratio of fluorescence intensities at the 567 nm and 539 nm (F567/F539) increased in response to changes in the $Zn^{2+}$ concentration (Fig. 3). When the $Zn^{2+}$ concentration was 10 μM (i.e., 10 eq.), F567/F539 was 8.0. The fluorescence color changed from greenish-yellow to orange. The sigmoidal plot shown in Fig. 3 gives a quantitative determination of $Zn^{2+}$. The LOD and LOQ of BDP-TPY for $Zn^{2+}$ were $5.1 \times 10^{-9}$ M and $1.7 \times 10^{-8}$ M, respectively. These values are sufficiently low for the detection in the submicromolar concentration range of $Zn^{2+}$ in many environmental, chemical, and biological systems.

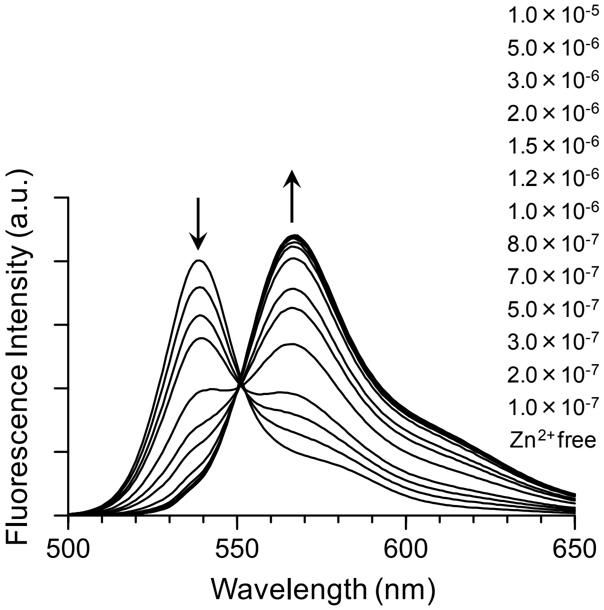

Fig. 2 Changes in the fluorescence spectrum of BDP-TPY with $Zn^{2+}$. The spectra are for $Zn^{2+}$ concentrations of 0, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0, 5.0, and 10.0 μM. Spectra were acquired in aqueous acetonitrile solution with excitation at 535 nm. The concentration of BDP-TPY was 1 μM.

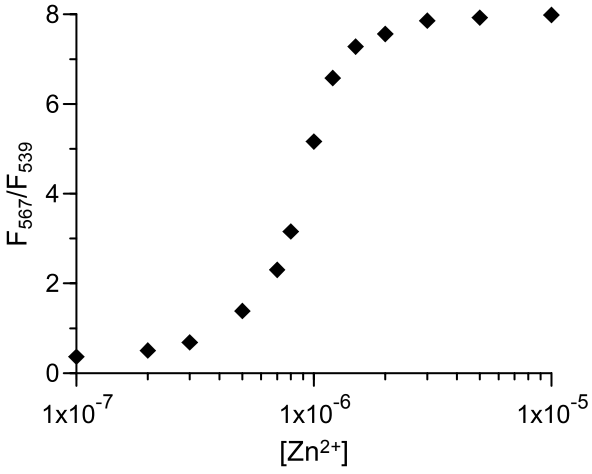

Fig. 3 Plot of the fluorescence intensity ratio (F567/F539) of BDP-TPY versus increasing $Zn^{2+}$ concentration.

Competition experiments of $Zn^{2+}$ with some common ions were conducted to evaluate the selectivity of BDP-TPY toward $Zn^{2+}$ (Fig. 4). The fluorescence intensity ratio (F567/F539) did not change upon addition of $Na^+$, $Mg^{2+}$, $K^+$, $Ca^{2+}$, $Cr^{3+}$, $Mn^{2+}$, and $Fe^{3+}$ to the solution of BDP-TPY. The signal increased slightly for $Hg^{2+}$ and $Pb^{2+}$ solutions, whereas it increased up to 5.2 for the $Cd^{2+}$ solution. Further addition of $Zn^{2+}$ to the solutions resulted in further increases in the signal, indicating that BDP-TPY preferentially binds to $Zn^{2+}$ rather than $Cd^{2+}$ or $Hg^{2+}$. Other researches also reported that terpyridine shows good affinity for $Zn^{2+}$ (Goze 2003). However, complexation of BDP-TPY with $Fe^{2+}$ or $Fe^{3+}$ showed fluorescence quenching to some extent, and $Cu^{2+}$ completely quenched the fluorescence of BDP-TPY.

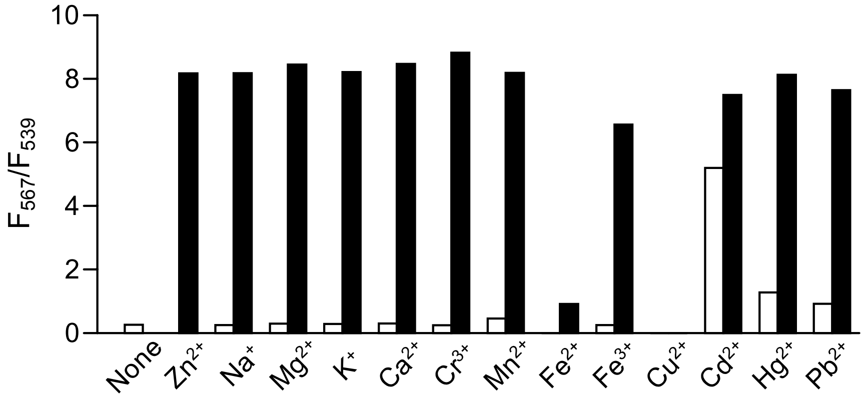

Figure 4. Changes of the fluorescence intensity ratio (F567/F539) of BDP-TPY upon addition of different metal ions. Spectra were acquired in aqueous acetonitrile solution. White bars represent the addition of an excess of the appropriate metal ion (1 mM for $Na^+$, $Mg^{2+}$, $K^+$, and $Ca^{2+}$. 10 μM for all other ions) to a 1 μM solution of BDP-TPY. Black bars represent the subsequent addition of 10 μM of $Zn^{2+}$ to the solution. Excitation was provided at 535 nm.

## CONCLUSION

Terpyridine was directly linked to the 3 position of the BODIPY core using the Suzuki-Miyaura cross-coupling reaction as a metal-ion receptor. The fluoroionophore (BDP-TPY) responded to several heavy-metal ions with specific fluorescence spectra that probably depend on the nature of the ionic species. $Zn^{2+}$ concentration was successfully determined using a ratiometric fluorescence method.

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