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A Hg^{2+} fluorescent chemosensor without interference from anions and Hg^{2+}-imaging in living cells

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ABSTRACT

A fluorescent chemosensor, **B2**, for Hg^{2+} containing a BODIPY fluorophore and carboxyl-thiol metal-bonding moieties was described. **B2** exhibits selective fluorescence enhancement (123-fold) toward Hg^{2+} over other metal ions. Especially, the fluorescence enhancement was unaffected by anions existing in environment and organism. **B2** shows high sensitivity to Hg^{2+} in a concentration of ppb range with detection limit of 77 nM. **B2-ester**, the membrane-permeable ethyl ester, is able to be hydrolyzed to **B2** *in vivo*, and successfully applied to image intracellular Hg^{2+} in living cells.

1. Introduction

Mercury is one of the most toxic and dangerous heavy metal elements [1]. Mercury contamination is widespread and occurs through various processes, e.g. volcanic emissions, mining, solid waste incineration, and the combustion of fossil fuels [2]. Of particular concern, is the concentration in the food chain, and bioaccumulation of mercury in animals [3]. It is frightening that mercury-containing chemicals have been linked with a number of human health problems, including minamata, myocardial infarction, and some kinds of autism, and can lead to damage of the brain, kidneys, central nervous system, immune system and endocrine system [4,5]. Thus, much attention has been focused on developing new methods to monitor Hg^{2+} in biological and environmental samples.

Recently, much effort has been made to design Hg^{2+} fluorescent sensors with high sensitivity and selectivity, quick response time and easy signal detection [6–9]. But only a few reports discussed the effect of anions on the fluorescence change toward Hg^{2+}. Most of these fluorescent chemosensors for Hg^{2+}, however, are affected by different anions [6f,7c,9c,10], especially by chloride ion. As an illustration of **B1** (Scheme 1) in our previous research [11], Cl^-, Br^-, CO_3^{2-}, SCN^- and CH_3COO^- could affect the fluorescence enhancement through formation of endo- or exo-metal complexes with Hg^{2+}. This is a fatal defect for practical applications such as the cases in environmental determinations and biological-detections, where anions are always co-existent with Hg^{2+}. Lin reported a sensor (DMABTS) (Scheme 1) independent from anions. The sensor, however, was a fluorescent quenching sensor [12]. Recently, Duan reported a fluorescence-enhancing and anion-independent chemosensor **RF1** [13]. The sensor combines a ferrocene unit and a rhodamine block via the linkage of a carbohydrazone (Scheme 1). As the bulky ferrocene is close to the receptor, an anion is resistant to access the spirolactam center which is sensitive to analyte. However, the bulky sensor is impenetrable to cells and could not be used in Hg^{2+} cellular imaging. Herein we report a new Hg^{2+}-fluorescence chemosensor, **B2**, which is selective for Hg^{2+}, independent from anions and permeable to living cells via its ethyl ester.

2. Experimental

2.1. Materials and apparatus

All the chemicals and solvents were of analytical reagents. The solutions of metal ions were prepared from the nitrate salts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Cr^{3+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Co^{2+}, Hg^{2+}, Ag^+, and the sodium salts of Cl^-(Br^-), HCO_3^-, CO_3^{2-}, SO_4^{2-}, ClO_4^-, CH_3COO^-, $H_2PO_4^-$, respectively. The different salts were then dissolved in distilled water. NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in

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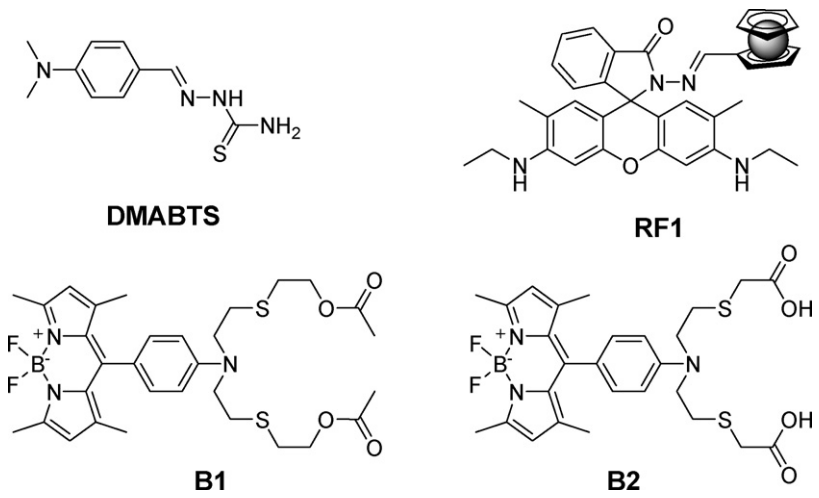

Scheme 1. Structures of Hg^{2+} sensors.

$CDCl_3$, TMS as internal standard). Mass spectral determinations were made on a HP1100 API-ES mass spectrometer. Fluorescence measurements were performed on a PTI-700 Felix and Time-Master system. Absorption spectra were measured on Lambda 35 UV/vis spectrophotometer. The pH measurement was recorded by PHS-SC instrument.

2.2. Synthesis of intermediates and probes

2.2.1. Synthesis of compound *3*

In a 25 mL flask, sodium (0.48 g, 20 mmol) is added to anhydrous ethanol (12 mL). After sodium was dissolved completely, ethyl-2-mercaptoacetate (2.40 g, 23 mmol) was added dropwise. The mixture was stirred for 2 h at 40 °C. Then a solution of 4-(bis(2-chloroethyl)amino)benzaldehyde (**2**) (2.24 g, 9 mmol) in DMF (5 mL) was added. The stirring was continued for another 3 h. Water (20 mL) was added to the residual and extracted with dichloromethane (3× 10 mL). The combined organic phase was washed twice with water and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation and dried in vacuum, yielding a yellow oil of compound **3** (3.52 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.28 (t, J = 7 Hz, 6H), 2.88 (t, J = 9 Hz, 4H), 3.28 (s, 4H), 3.69 (t, J = 8 Hz, 4H), 4.20 (m, 4H), 6.75 (d, J = 9 Hz, 2H), 7.74 (d, J = 9 Hz, 2H), 9.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 189.55, 169.86, 151.19, 131.84, 125.54, 110.78, 61.10, 50.30, 33.17, 28.98, 13.85; TOF MS(ES): m/z Calcd for 414.1409 ($M+H^+$), Found: 414.1418.

2.2.2. Synthesis of ***B2-ester***

400 mg (1 mmol) of **3** and 0.3 mL of 2,4-dimethylpyrrole were dissolved in 150 mL of absolute dichloromethane under nitrogen atmosphere, one drop of trifluoroacetic acid was added and the solution was stirred at room temperature for 5 h. After the mixture was concentrated to 40 mL, a solution of tetrachlorobenzoquinone (0.5 g, 2 mmol) in 10 mL of dichloromethane was added and stirring was continued for 15 min, followed by the addition of triethylamine (2 mL) and $BF_3{\cdot}OEt_2$ (4 mL). After stirring for another 30 min, the reaction mixture was washed with 50 mL of water, extracted with dichloromethane (3× 20 mL). The extract was dried over anhydrous magnesium sulfate and then concentrated under vacuum. The product was purified by flash column chromatography using petrol ether/ethyl acetate (3:1, v/v) as eluant, yielding a brown oil of **B2-ester** (80 mg, 13%). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.29 (t, J = 7 Hz, 6H), 1.48 (s, 6H), 2.54 (s, 6H), 2.89 (t, J = 8 Hz, 4H), 3.27 (s, 4H), 3.64 (t, J = 7 Hz, 4H), 4.20 (m, 4H), 5.97 (s, 2H), 6.78 (d, J = 8 Hz, 2H), 7.07 (d, J = 9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 170.32, 154.86, 147.13, 143.11, 142.66, 132.09, 129.23, 122.82, 120.92, 112.07, 77.36, 77.25, 77.05, 76.73, 61.53, 50.78, 33.61, 29.44, 14.70, 14.56, 14.20; TOF MS (ES): m/z Calcd for 654.2419 ($M+Na^+$), Found: 654.2435.

2.2.3. Synthesis of ***B2***

A solution of **B2-ester** (43 mg, 0.07 mmol) in a 1:1 tetrahydrofuran/methanol mixture (10 mL) under a nitrogen atmosphere was cooled to 0 °C and treated with 15 equiv. of lithium hydroxide. The resulting mixture was stirred for 2 days under nitrogen at which time TLC analysis showed complete consumption of starting material. Water (15 mL) was added to the resultant and the solution was made acidic (pH 6) by the addition of hydrochloric acid. The mixture was extracted with dichloromethane. The organic fractions were combined and dried over anhydrous magnesium sulfate, and the solvent was removed in vacuum to give the product. The product was purified by flash column chromatography using dichloromethane/methanol (10:1, v/v) as eluant, yielding a brown oil of **B2** (35 mg, 89%). TOF MS (ES): m/z Calcd for 574.1817 ($M-H^+$), Found: 574.1823.

2.3. Cell culture and labeling of cells

PC12 cells were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). One day before imaging, cells were placed in 24-well flat-bottomed plates. The next day, parts of the PC12 cells were incubated with 10 μM **B2-ester** for 30 min at 37 °C under 5% CO_2 as a control. The rest PC12 cells were incubated with 10 μM $Hg(ClO_4)_2$ for 30 min at 37 °C and then washed with phosphate-buffered saline (PBS) before incubating with 10 μM **B2-ester**. Finally, cells in 24-well plates were rinsed with PBS three times again, then the fluorescence imaging of intracellular Hg^{2+} was observed under Nikon eclipase TE2000-5 inverted fluorescence microscopy. For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular Hg^{2+} fluorescence.

3. Results and discussion

3.1. Synthesis

The synthetic route for **B2** is shown in Scheme 2. Reaction of *N*,*N*-bis(2-hydroxyethyl)aniline (**1**) with $POCl_3$/DMF followed by basic treatment generated 4-(bis(2-chloroethyl)amino)benzaldehyde (**2**) in 58% yield. Compound **2** was further reacted with 2-