J|A|C|S
COMMUNICATIONS

Published on Web 01/19/2007

A Selective Fluorescent Sensor for Imaging Cd^{2+} in Living Cells

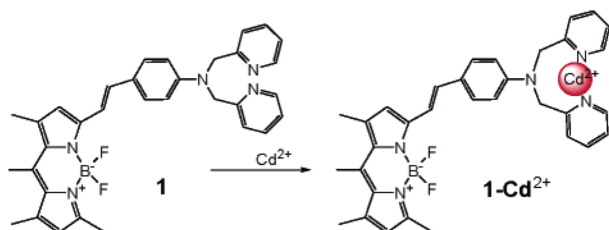
Xiaojun Peng,* Jianjun Du, Jiangli Fan,* Jingyun Wang, Yunkou Wu, Jianzhang Zhao, Shiguo Sun, and Tao Xu

State Key Laboratory of Fine Chemicals, Dalian University of Technology, 158 Zhongshan Road, Dalian 116012, P. R. China

Received June 19, 2006; E-mail: pengxj@dlut.edu.cn; fanjl@dlut.edu.cn

Cadmium is currently used in many processes such as electroplating, metallurgy, war industry, etc., and it is even found in phosphate fertilizers.[1] These sources lead to cadmium exposure, and in certain areas, there is evidence of increasing cadmium content in food, which poses severe harm for human health and the environment.[2] There have been many reports on the toxicity of Cd^{2+} to procreation, bones, kidneys, nerve system, and tissues, consequently resulting in renal dysfunction, calcium metabolism disorders, and an increased incidence of certain forms of cancers.[3] Since cadmium can be accumulated in organisms, there is a great need for methods of detecting and monitoring cadmium levels in living cells or tissue samples.

Fluorescent sensors are often used to detect many ions owing to their simplicity and sensitivity.[4] However, only a few examples of fluorescent sensors for Cd^{2+} have been reported.[5] Generally, Cd^{2+} and Zn^{2+} have very similar chemical properties, so the discrimination between them is very difficult.[6] Recently, Gunnlaugsson et al. have reported the first example of a Cd^{2+}-selective fluorescent chemosensor, which can distinguish Cd^{2+} from Zn^{2+} to some extent by their different bathochromic shift of the fluorescence spectra.[7] Unfortunately, up to now, there is no report about a Cd^{2+}-selective sensor suitable in living cells. Herein we describe the first intracellular emission fluorescent Cd^{2+} sensor **1** based on the ICT mechanism.



The internal charge transfer (ICT) mechanism[8] has been widely exploited for ions sensing,[9] molecular switching,[10] and fluorescent labeling[11] due to the advantages of spectral shifts and quantitative detection. When a fluorophore contains an electron-donating group (often an amino group) conjugating to a fluorophore, it undergoes ICT from the donor to the fluorophore upon light excitation, which provides a red-shifted emission. Coordinated with an ion, the amino group loses its donating ability. Consequently, the ICT is inhibited and the emission blue shifts. Fluorescence quantum yields always change in the processes. In sensor **1**, we chose boradiazaindacene (BODIPY) as the fluorophore because it absorbs and emits in the visible region with high quantum yield, large extinction coefficient, and good photostability[12] and *N*,*N*-bis(pyridin-2-ylmethyl)benzenamine as Cd^{2+} receptor (and ICT donor). A vinyl group between the receptor and the BODIPY fluorophore can induce longer wavelengths in absorption and fluorescence spectra.

Sensor **1** was synthesized according to the general route[10] by condensation of BODIPY (4,4-difluoro-1,3,4,5,7-quantmethyl-4-bora-3a,4a-diaza-*s*-indacene) with 4-(bis(pyridin-2-ylmethyl)amino)-benzaldehyde in 18% yield.

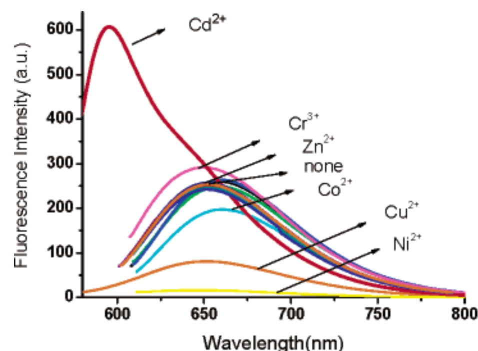


Figure 1. The fluorescence spectra of **1** (5 μM) in the presence of different metal ions (150 μM) in Tris-HCl (0.01 M) solution (acetone/water = 9/1, v/v, pH 7.4), and nearly no response to some other metal ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Hg^{2+}, Ag^{+}, Mn^{2+}, Pb^{2+}).

The λ_{max}ab in the absorption spectra of free **1** in the ICT band is near 600 nm (Figure S1a in Supporting Information). When Cd^{2+} was added gradually, the λ_{ab} showed a 29 nm blue shift with an isosbestic point at 580 nm and the color of the solution turned from light blue to bright pink (Figure S8 in Supporting Information).

In the fluorescence emission, free **1** exhibits λ_{max}em at 656 nm with a quantum yield of 0.12. Upon addition of $CdCl_2$, the λ_{max}em undergoes a blue shift to 597 nm with a quantum yield of 0.59 (Figure 1). A well-defined isoemission point at 673 nm is also observed (Figure S1b). The emission intensity at 597 nm (F_{597}, Figure S2) and the intensity ratio, R (F_{597}/F_{697}, Figure S3), increased upon the gradual addition of Cd^{2+}, which allowed the detection of Cd^{2+} by both normal fluorescence and ratiometric fluorescence methods. From the sigmoidal curves in Figures S2 and S3, dissociation constants $(4.8 \pm 0.3) \times 10^{-5}$ and $(7.0 \pm 0.3) \times 10^{-5}$ M are obtained, respectively. The fluorescence responses in both methods fit a Hill coefficient of 1 (Figures S4 and S5); it is consistent with the formation of a 1:1 stoichiometry for the **1**$-Cd^{2+}$ complex.

The fluorescence titration of **1** with various metal ions (Figure 1) shows excellent selectivity to Cd^{2+}. Physiologically important metal ions which exist in living cells, such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and Fe^{3+}, do not give any responses at 30-fold excess concentration. Most heavy and transition metal ions, such as Hg^{2+}, Ag^{+}, Mn^{2+}, and Zn^{2+}, also have no interference. Only Cr^{3+} induces very slight fluorescence enhancement. Ni^{2+} and Cu^{2+} obviously quench the fluorescence to some extent, which always meet in the other metal ion sensors.[13]

The competition experiments of Cd^{2+} mixed with the metal ions show no significant variation in the ratio fluorescence intensity (F_{597}/F_{697}, Figure 2), although Cu^{2+} and Ni^{2+} have some fluorescence quenching in normal fluorescence intensity at 597 $\pm$ 15 nm (F_{597}, Figure S6). Another Zn^{2+} titration experiment suggests that Zn^{2+} cannot induce any response of **1** even at high concentrations

10.1021/ja0643319 CCC: $37.00 © 2007 American Chemical Society

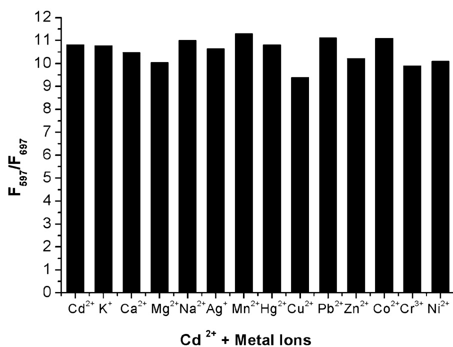


Figure 2. The ratio fluorescence responses (F_{597}/F_{697}) of sensor **1** containing 250 μM Cd^{2+} to the selected metal ions (250 μM) in Tris-HCl (0.01 M) solution (acetone/water, 9/1, v/v, pH 7.4). The concentration of **1** was 5 μM, and excitation wavelength was 580 nm.

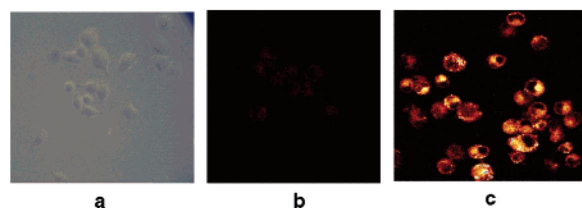


Figure 3. Confocal fluorescence images of Cd^{2+} in DC cells. The excited light is 543 nm, and the emission is centered at 597 ± 15 nm (Leica TCS-SP2 confocal fluorescence microscope, 20× objective lens). (a) Bright-field transmission image of DC cells incubated with **1** (5 μM). (b) Fluorescence image of DC cells incubated with **1** (5 μM). (c) Fluorescence image of DC cells incubated with **1** for 30 min, washed three times, and then further incubated with 5 μM $CdCl_2$ for 30 min.

(Figures S7 and S8). To the best of our knowledge, this is the first example of fluorescent Cd^{2+} sensors which can distinguish Cd^{2+} from Zn^{2+} with both emission shift and fluorescence intensity.

Fluorescent sensors based on electron donor/acceptor are usually disturbed by a proton in the detection of metal ions. **1** displays intense fluorescence at pH < 4. When the pH is >5.5, however, the fluorescence intensities are very low and remain constant. The pK_a is 4.1 from the sigmoidal curve (Figure S9). Therefore, sensor **1** can be used in the aqueous media with pH > 5.5.

To determine the cell permeability of **1**, PC12 cells were incubated with **1** (5 μM). The increases in the fluorescence intensity in living cells were observed upon addition of Cd^{2+} (5 μM) into the medium and incubation for 0.5 h at 37 °C. The images were obtained on a Nikon Eclipse TE2000-5 fluorescence microscope excited by its green light (510−560 nm) (Figure S10). Although the microscope recorded a wide emission wavelength range (580−700 nm), the penetrating ability and the intracellular Cd^{2+} sensing of **1** are very clear.

Similarly to PC12 cells, DC cells were incubated with **1** (5 μM) and then further incubated with 5 μM $CdCl_2$. The fluorescence images of intracellular Cd^{2+} were observed under a Leica TCS-SP2 confocal microscope. The single-channel confocal fluorescence at 597 ± 15 nm (Figure 3) shows more clear images than that of a general microscope (Figure S10). The double-channel fluorescence images at 597 ± 15 and 697 ± 15 nm are shown in Figure S11. Analyzed by MetaFluor software (Universal Imaging Corp.), ratio fluorescence images were obtained (Figure 4).

The results suggest that **1** can be used to image intracellular Cd^{2+} in living cells in both general fluorescence and ratio fluorescence ways. It should therefore be potentially useful for the study of the toxicity or bioactivity of Cd^{2+} in living cells.

In conclusion, we have reported that fluorescent sensor **1** can be used for selective imaging of Cd^{2+} in living cells. It can

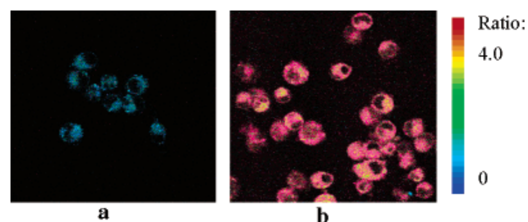


Figure 4. Ratio fluorescence (F_{597}/F_{697}) images of Cd^{2+} in DC cells (Leica TCS-SP2 confocal fluorescence microscope, 20× objective lens). (a) DC cells incubated with **1** (5 μM). (b) DC cells incubated with **1** and then further incubated with 5 μM $CdCl_2$.

distinguish Cd^{2+} from Zn^{2+} and especially it can be used in both general fluorescence microscopy and ratiometric fluorescence microscopy.

Acknowledgment. This work was supported by the National Science Foundation of China (20376010 and 20472012).

Supporting Information Available: Synthesis, experimental details, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chaney, R. L.; Ryan, J. A.; Li, Y.-M.; Brown, S. L. In *Cadmium in Soils and Plants*; McLaughlin, M. J., Singh, B. R., Eds.; Kluwer: Boston, 1999; pp 219−256.
(2) Dobson, S. *Cadmium: Environmental Aspects*; World Health Organization: Geneva, 1992.
(3) Friberg, L.; Elinger, C. G.; Kjellström, T. *Cadmium*; World Health Organization: Geneva, 1992.
(4) (a) Qi, X.; Jun, E. J.; Xu, L.; Kim, S.-J.; Joong Hong, J. S.; Yoon, Y. J.; Yoon, J. *J. Org. Chem.* **2006**, *71*, 2881−2884. (b) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270−14271. (c) Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16030−16031. (d) Hirano, T.; Kikuchi, K.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 6555−6562. (e) Wu, Y.; Peng, X.; Guo, B.; Fan, J.; Zhang, Z.; Wang, J.; Cui, A.; Gao, Y. *Org. Biomol. Chem.* **2005**, *3*, 1387−1392.
(5) (a) Huston, M. E.; Engleman, C.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 7054−7056. (b) Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Eur. J. Inorg. Chem.* **1999**, *3*, 455−460. (c) Prodi, L.; Montalti, M.; Zaccheroni, N.; Bradshaw, J. S.; Izatt, R. M.; Savage, P. B. *Tetrahedron Lett.* **2001**, *42*, 2941−2944. (d) Choi, M.; Kim, M.; Lee, K. D.; Han, K. N.; Yoon, I. A.; Chung, H. J.; Yoon, *J. Org. Lett.* **2001**, *3*, 3455−3457. (e) Charles, S.; Yunus, S.; Dubois, F.; Vander Donckt, E. *Anal. Chim. Acta* **2001**, *440*, 37−43. (f) Marino, J. E.; Resendiz, J. C.; Disteldorf, N. H.; Fischer, S.; Stang, P. J. *Org. Lett.* **2004**, *6*, 651−653. (g) Bronson, R. T.; Michaelis, D. J.; Lamb, R. D.; Husseini, G. A.; Farnsworth, P. B.; Linford, M. R.; Izatt, R. M.; Bradshaw, J. S.; Savage, P. B. *Org. Lett.* **2005**, *7*, 1105−1108.
(6) (a) Cotton, F. A.; Wilkinson, G. *Advances in Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; pp 957−1358. (b) Dakternieks, D. *Coord. Chem. Rev.* **1990**, *98*, 279−294.
(7) (a) Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Org. Lett.* **2003**, *5*, 4065−4068. (b) Gunnlaugsson, T.; Lee, T. C.; Parkesh, R. *Tetrahedron* **2004**, *60*, 11239−11249.
(8) (a) de Silva, A. P.; Nimal Gunaratne, H. Q.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (b) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3−40. (c) Grabowski, Z. R.; Rotkiewicz, K. *Chem. Rev.* **2003**, *103*, 3899−4031.
(9) (a) Maruyama, S.; Kikuchi, K.; Hirano, T.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 10650−10651. (b) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712−713. (c) Xu, Z.; Qian, X.; Cui, J. *Org. Lett.* **2005**, *7*, 3029−3032. (d) Coskun, A.; Ankara, E. U. *J. Am. Chem. Soc.* **2005**, *127*, 10464−10465. (e) Baruah, M.; Qin, W.; Vallee, R. A. L.; Beljonne, D.; Rohand, T.; Dehaen, W.; Boens, N. *Org. Lett.* **2005**, *7*, 4377−4380. (f) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *J. Am. Chem. Soc.* **2005**, *127*, 3635−3641. (g) Kiyose, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 6548−6549.
(10) (a) Rurack, K.; Kollmannsberger, M.; Daub, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 385−387. (b) Coskun, A.; Deniz, E.; Akkaya, E. U. *Org. Lett.* **2005**, *7*, 5187−5189.
(11) Peng, X.; Song, F.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J.; Gao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 4170−4171.
(12) Karolin, J.; Johansson, L. B.-A.; Strandberg, L.; Ny, T. *J. Am. Chem. Soc.* **1994**, *116*, 7801−7806.
(13) Zheng, Y.; Orbulescu, J.; Ji, X.; Andreopoulos, F. M.; Pham, s. M.; Leblanc, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 2680−2686.

JA0643319