Turk J Chem
35 (2011) , 475 – 479.

doi:10.3906/kim-1007-830

A near-infrared fluorescent sensor for H^+ in aqueous solution and living cells

Aibin WU[1,2], **Liping DUAN**[1,*]
[1]*National Institute for Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, 200025, P. R. CHINA*
e-mail: lipingduan4@hotmail.com
[2]*School of Chemistry and Environmental Engineering, Yangtze University, Jingzhou, 434023, P. R. CHINA*
e-mail: abwu@yangtzeu.edu.cn

Received: 29.07.2010

A heptamethine cyanine-based sensor (**1**) was designed and synthesized by incorporating heptamethine cyanine fluorophore and methylpiperazine. Sensor **1** exhibited good response to the change of pH levels, and a large Stokes shift (>100 nm) was obtained. Fluorescent image experiments in living cells further demonstrated its potential applications in biological systems.

Key Words: Fluorescent image, heptamethine cyanine, pH sensor

Introduction

In light of the important roles of pH in a variety of fundamental physiological processes in organisms, the study of selective and sensitive methods for pH detection has gained tremendous attention.[1–7] However, most of the commercially available pH sensors are known to emit at between 350 and 600 nm. It has been proven that this spectral region suffers from not only strong background absorbance interference but also autofluorescence from the biological environment or endogenous chromophores in sample media,[8–12] which limits their use as biological sensors or as reagents for photodynamic therapy. Therefore, near-infrared heptamethine cyanine dyes, which are employed as fluorescent labels and sensors of biomolecules, have attracted immense interest in the past decades.[13–17] Nevertheless, most of the polymethine cyanine dyes have had the fatal disadvantage of their Stokes shifts being less than 25 nm.[18,19] A small Stokes shift could result in self-quenching and measurement

*Corresponding author

error by excitation and scattering light. It is obvious that near-infrared cyanine dye with a larger Stokes shift would have promising applications in fluorescent bioassays. Furthermore, the dyes should be nontoxic and water-soluble and should have a pK_a value within the physiological pH range (generally between 6 and 8).[20] Bearing these points in mind, we designed and synthesized heptamethine cyanine-based sensor **1,** containing 2 sulfonate groups that could provide better water solubility when they were applied to biological analysis in an aqueous environment.

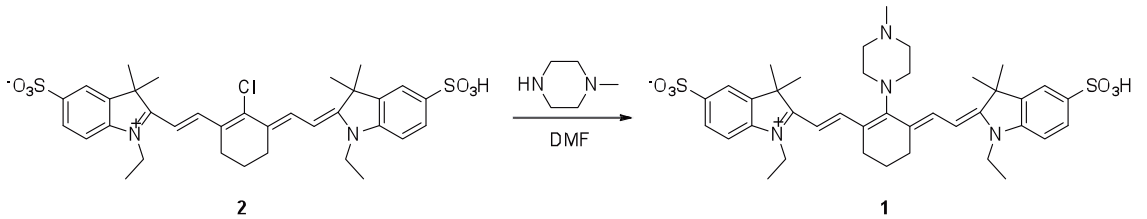
Experimental

Reagents and apparatus

All reagents and solvents were commercially available and used without further purification. ^{1}H-NMR spectra were measured on a Bruker AM-400 spectrometer with chemical shifts reported in ppm (in DMSO-d_6, TMS as the internal standard). Mass spectra were obtained on an HP 5989A spectrometer. Fluorescent spectroscopic studies were performed with a Varian Cary Eclipse. Absorption spectra were recorded on a Varian Cary 100 spectrophotometer. pH was measured with a Sartorius PB-10 pH meter. Fluorescent images were determined on a Leica DM IRB instrument.

Procedure

The synthetic route of designed heptamethine cyanine dye **1** is shown in Scheme 1. Compound **2** (68 mg, 0.01 mmol), purchased from Sigma Aldrich, was dissolved in DMF (2 mL), and then *N*-methylpiperazine (100 mg, 0.1 mmol) was added. The solution was stirred under nitrogen at room temperature for 2 h and then concentrated under vacuum. The residue was subjected to column chromatography on silica gel by using CH_3OH/CH_2Cl_2 (1:10 v/v) as an eluent, affording sensor **1** (40 mg, 54%) as a dark blue solid. Mp 191.1-192.3 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 7.66 (d, J = 14 Hz, 2H), 7.56-7.59 (m, 4H), 7.13 (d, J = 7.8 Hz, 2H), 5.90 (d, J = 14 Hz, 2H), 4.04 (t, J = 7.2 Hz, 4H), 3.73 (t, J = 7.2 Hz, 4H), 2.62 (t, J = 7.2 Hz, 4H), 2.31 (t, J = 5.7 Hz, 4H), 2.07 (s, 3H), 1.75 (m, 2H), 1.61 (s, 12H), 1.22 (t, J = 7.2 Hz, 6H); HRMS (ES) calcd for $C_{39}H_{50}N_4O_6S_2$ $([M-H])^+$: 733.3632, found: 733.3636.



Scheme 1. Synthetic route of heptamethine cyanine dye **1**

V79 379A Chinese hamster cells were used for fluorescent image analysis of sensor **1**. Briefly, cells from exponentially growing cultures were seeded in a 24-well culture plate and treated with sensor **1** (10 μM) for 2 h at 37 °C, then examined with a Leica DM IRB fluorescent microscope.

Results and discussion

Response of sensor 1 to H^+ in absorption spectra

The absorption spectra of sensor **1** and its H^+ titration were determined in H_2O. As shown in Figure 1 (left), the absorption intensity at 700 nm decreased with the concomitant formation of a band that peaked at 665 nm upon addition of H^+, which exhibited a large blue shift (35 nm) and a large Stokes shift (>100 nm). Moreover, a clear isosbestic point at 680 nm was observed, indicating that compound **3** was formed by the reaction of sensor **1** with H^+ (Scheme 2).

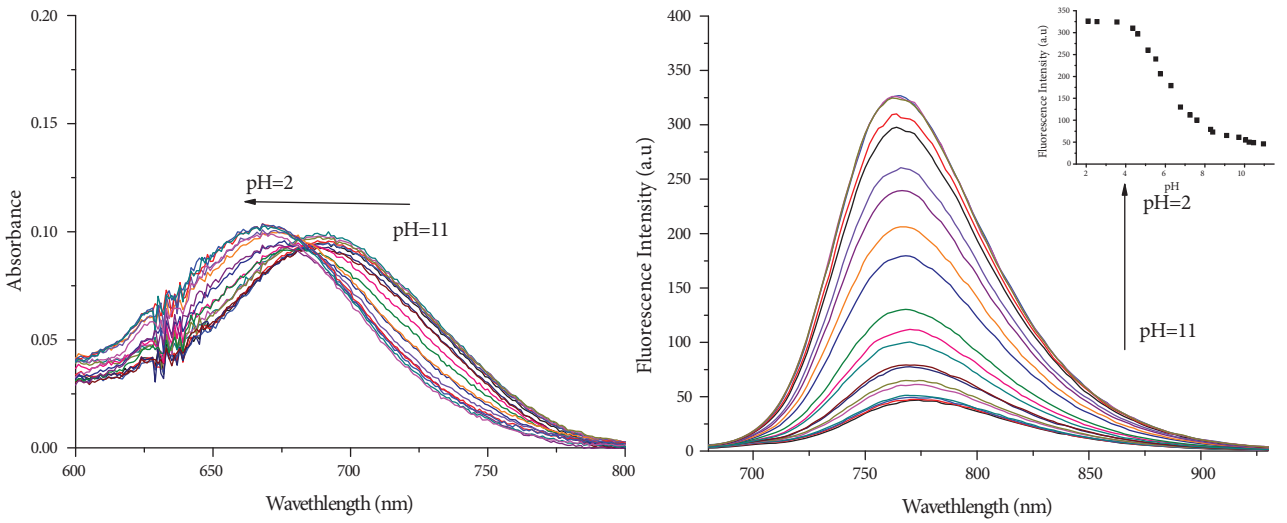
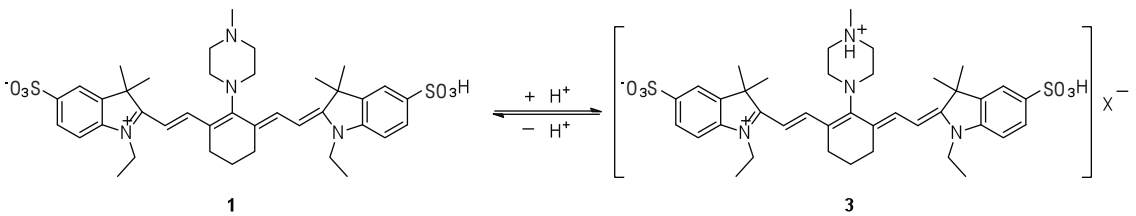


Figure 1. Influences of pH on the absorption (left) and fluorescence (right) of sensor **1** in water. The pH was modulated by adding 75% $HClO_4$ or NaOH solution. Inset: curve of fluorescence intensity (I) versus pH.



Scheme 2. Proposed binding mode between sensor **1** and H^+.

Response of sensor 1 to H^+ in emission spectra

Fluorescent properties of sensor **1** are displayed in Figure 1 (right). Just as expected, the nitrogen atom in the molecule was protonated at low pH levels, lower than the pK_a values, such that the fluorescence intensity was enhanced. This was due to the depression of the photoinduced electron transfer (PET) process. We found that the fluorescence intensity at a low pH level (2.43) was 7-fold larger than that at a high pH level (9.80). Furthermore, the fluorescence changes were fully reversible and took place mainly within the pH range of 2.43–9.80, which indicated that the I/I_{max} of sensor **1** was a function of pH, where I was the measured fluorescence

intensity at that pH and I_{max} was the maximum output of the sensor. A regular sigmoidal curve was observed for the sensor in response to pH. The pK_a of sensor **1** was calculated as 6.34 where I/I_{max} was 0.55 (Figure 2).

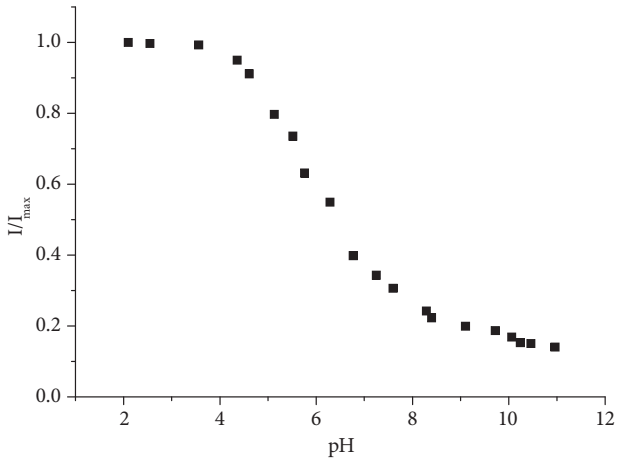


Figure 2. pK_a calculation of sensor **1** according to the curve of I/I_{max} versus pH.

Fluorescent image application

To further demonstrate the practical application of sensor **1**, we carried out an experiment for H^+ detection in living cells. After incubation of V79 379A cells with sensor **1** (10 μM) at 37 °C for 1 h in different pH environments, different enhancements of fluorescence were observed (Figure 3). In order to obtain clear images, the experiments were carried out at pH 6 and 8; the cells could not have survived at extreme pH levels. The results suggested that sensor **1** could not only penetrate the cell membrane and be used for imaging of H^+ in living cells, but could also be used for detecting the changes of H^+ in biological systems.

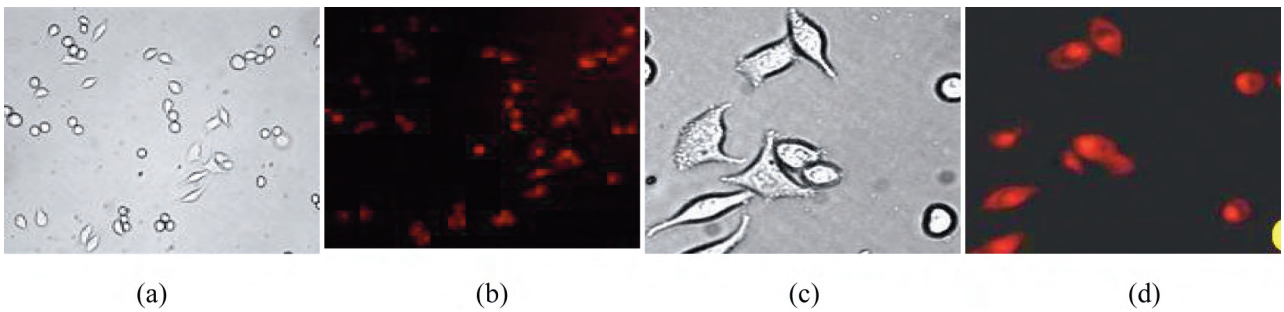


Figure 3. Fluorescent images of H^+ in V79 379A Chinese hamster cells: a) bright-field transmission image of cells at pH 8, b) fluorescent image of cells at pH 8, c) bright-field transmission image of cells at pH 6, d) fluorescent image of cells at pH 6. Images were taken after incubation with sensor **1** for 1 h at different pH levels.

Conclusions

In conclusion, we have developed a new fluorescent sensor (**1**) based on a heptamethine cyanine fluorophore. It showed high sensitivity toward H^+ in aqueous solution, and the fluorescence intensity was significantly enhanced

by about 7-fold from pH 9.80 to 2.43. Moreover, it exhibited a large Stokes shift (>100 nm). The fluorescent image in a living cell further demonstrated its practical applications in a biological system.

Acknowledgements

The authors wish to acknowledge that this project was supported by the Chinese Center for Disease Control and Prevention (2010A102), the Shanghai Health Bureau, and the Science Research Project of Hubei Provincial Department of Education (Q20111304), and the Science Research and Development Foundation of Yangtze University.

References

1. Uchiyama, S.; Makino, Y. *Chem. Commun.* **2009**, *19*, 2646-2648.
2. Bojinov, V. B.; Georgiev, N. I.; Bosch, P. *J. Fluores.* **2009**, *19*, 127-139.
3. Lee, H.; Berezin, M. Y.; Guo, K.; Kao, J.; Achilefu, S. *Org. Lett.* **2009**, *11*, 29-32.
4. Ooyama, Y.; Egawa, H.; Yoshida, K. *Dyes and Pigments* **2009**, *82*, 58-64.
5. Shimoda, L. A.; Luke, T.; Sylvester, J. T.; Shih, H. W.; Jain, A.; Swenson, E. R. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2007**, *292*, 1013-1022.
6. Kang, M.; Walker, J. W. *Exp. Biol. Med.* **2006**, *231*, 865-870.
7. Klonis, N.; Sawyer, W. H. *J. Fluoresc.* **1996**, *6*, 147-157.
8. Rurack, K.; Kollmannsberger, M.; Daub, J. *Angew. Chem. Int. Ed.* **2001**, *40*, 385-387.
9. Mustroph, H.; Stollenwerk, M.; Bressau, V. *Angew. Chem. Int. Ed.* **2006**, *45*, 2016-2035.
10. Zhang, Z. R.; Achilefu, S. *Chem. Commun.* **2005**, *47*, 5887-5889.
11. Urano, Y.; Asanuma, D.; Hama, Y.; Koyama, Y.; Barret, T.; Kamiya, M.; Nagano, T.; Watanabe, T.; Hasegawa, A.; Choyke, P. L.; Kobayashi, H. *Nature. Med.* **2009**, *15*, 104-109.
12. Lu, H.; Xu, B.; Dong, Y.; Chen, F.; Li, Y.; Li, Z.; He, J.; Li, H.; Tian, W. *Langmuir* **2010**, *26*, 6838-6844.
13. Briggs, M. S.; Burns, D. D.; Cooper, M. E.; Gregory, S. J. *Chem. Commun.* **2000**, *23*, 2323-2324.
14. Cooper, M. E.; Gregory, S.; Adie, E.; Kalinka, S. *J. Fluoresc.* **2002**, *12*, 425-429.
15. Patonay, G.; Antoine, M. D. *Anal. Chem.* **1991**, *63*, 321A-327A.
16. Peng, X.; Song, F.; Lu, E.; Wang, Y.; Zhou, W.; Fan, J.; Gao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 4170-4171.
17. Zhou, L. C.; Liu, J. Y.; Zhao, G. J.; Shi, Y.; Peng, X. J.; Han, K. L. *Chem. Phys.* **2007**, *333*, 179-185.
18. Tolosa, L.; Nowaczyk, K.; Lakowicz, J. *An Introduction to Laser Spectroscopy*, 2nd edition, Kluwer, New York, 2002.
19. Zhang, Z.; Achilefu, S. *Org. Lett.* **2004**, *6*, 2067-2070.
20. Haugland, R. P. *Handbook of Fluorescent Probes*, Molecular Probes, Inc., Oregon, USA, 1999.