Bioorganic & Medicinal Chemistry Letters 20 (2010) 6708–6711

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

An amphiphilic fluorescent probe for the visualization of histamine in living cells

Daisuke Seto, Nobuaki Soh, Koji Nakano, Toshihiko Imato *

Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744, Moto-oka, Nishi-ku, Fukuoka 819-0395, Japan

ARTICLE INFO

Article history:
Received 9 April 2010
Revised 30 August 2010
Accepted 1 September 2010
Available online 8 September 2010

Keywords:
Fluorescence imaging
Histamine
Ligand exchange
Nile Red
Sensor molecule

ABSTRACT

A novel fluorescent probe composed of two moieties, Nile Red and an iminodiacetic acid–Ni^{2+} complex, for the detection of histamine in living cells is described. The probe was successfully applied to visualizing histamine in RAW264 cells, representing the first demonstration of the imaging of histamine itself in living cells.

It is well known that histamine is an essential factor for maintaining physiological homeostasis in humans and animals. It has also been reported to be involved in pathological processes, such as acute allergies, the immune system, inflammation, and gastric ulcers, as well as in mast cells, basophils, and the brain, where it functions as a neurotransmitter.[1] For example, it has been reported that an elevation in the concentration of Ca^{2+} ions in the intracellular phase by antigen stimulation results in the release of histamine from basophils[2] and, in the central nervous system, hypothalamic histamine plays very important roles in various biological phenomena.[3] Therefore, the direct visualization of histamine in living cells may help in the elucidation of currently unknown neuropharmacological functions of histamine and enable the development of antihistamine drugs. However, it is difficult to visualize the behavior of histamine in living cells directly in real-time using conventional methods, such as high-performance liquid chromatography (HPLC),[4] enzymatic assays,[5] flow immunoassay,[6] and spectrofluorimetry.[7] We wish to report herein, for the first time, on a new method for visualizing histamine in living cells. The method involves the use of novel fluorescent probes, Nile Red derivatives **1**-M^{2+} (where M^{2+} = Cu^{2+} and Ni^{2+}). These probes contain an iminodiacetic acid (IDA)–M^{2+} complex (a hydrophilic moiety) and Nile Red dye (a hydrophobic moiety). Nile Red is only sparingly soluble in water and has a tendency to localize in the form of intracellular lipid droplets because of its lipophilic characteristics, although it is cell membrane permeable when used in staining cells.[8] Thus, the probes developed in this study would be expected to be water-soluble and would be present throughout the cytoplasm due to the presence of the IDA moiety. Thus, it can be assumed that the probes would be broadly applicable to various cells and organs without the need for any special reagents[9] to enhance its permeation through a cell membrane or premodification for cell loading.[10]

The strategy for the detection of histamine using the probe is based on a ligand exchange reaction[11] between histamine and iminodiacetic acid in the probe on the M^{2+} ion, which has been discussed in our previous letter (Fig. 1).[12] M^{2+} ions function to quench[13] the fluorescence of Nile Red, while the M^{2+} ion in the probe becomes dissociated from the probe on coordination with histamine molecules. When this dissociation occurs, the fluorescent property of the probe is recovered. The efficiency of quenching by metal ions is dependent on the distance between the fluorophore and the quencher.[14] To maximize quenching efficiency by the M^{2+} ion, we utilized the Mannich reaction[15] to synthesize the probe, because this reaction results in a complex in which the Nile Red moiety is in close proximity to the iminodiacetic acid moiety. The close proximity of the Nile Red moiety to the iminodiacetic acid moiety would be expected to enhance the sensitivity of the system.

To identify an optimal metal ion for our probe **1**, solutions of various metal ions (Co^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, and Zn^{2+}) were added to equimolar solutions of probe **1**, and solutions containing an excess of histamine were then added to the above solutions. The fluorescent intensity of probe **1** was greatly decreased when Cu^{2+} and Ni^{2+} solutions were added and was subsequently recovered when the

* Corresponding author. Tel./fax: +81 92 802 2889.
E-mail address: imato@cstf.kyushu-u.ac.jp (T. Imato).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.003

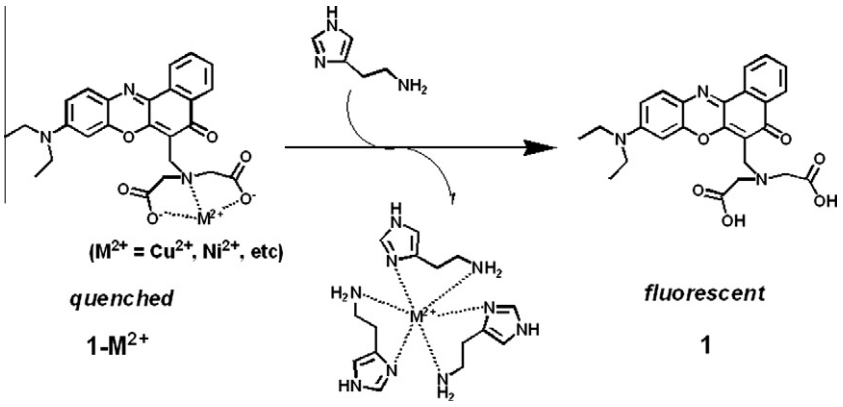

Figure 1. Reaction of **1**-M^{2+} with histamine.

histamine solution was added. Such a remarkable change in fluorescence intensity was not observed in the case of other metal ions (Fig. S1, Fig. S2, and Table S1 in the Supplementary data). Thus, we selected **1**-Cu^{2+} and **1**-Ni^{2+} as candidates for use in the histamine probe.

It is particularly important to evaluate the selectivity of **1**-Cu^{2+} and **1**-Ni^{2+} for histamine over other biogenic amines and glutathione because a wide variety of amines and thiols are present in living cells. Indeed, our probes were added to solutions of some selected amines and a thiol, including cadaverine, dopamine, GABA, glutamic acid (Glu), glutathione, glycine (Gly), putrescine and serotonin as well as a histamine solution to evaluate the selectivity of the probe (Fig. 2). As shown in Figure 2b, 1-Cu^{2+} showed a rather low selectivity to histamine compared with the fluorescence intensity for dopamine, Glu, glutathione, and Gly. In particular, the fluorescence intensity of **1**-Cu^{2+} in the presence of glutathione was comparable to that observed for the addition of histamine. The higher response of glutathione compared to other amines except

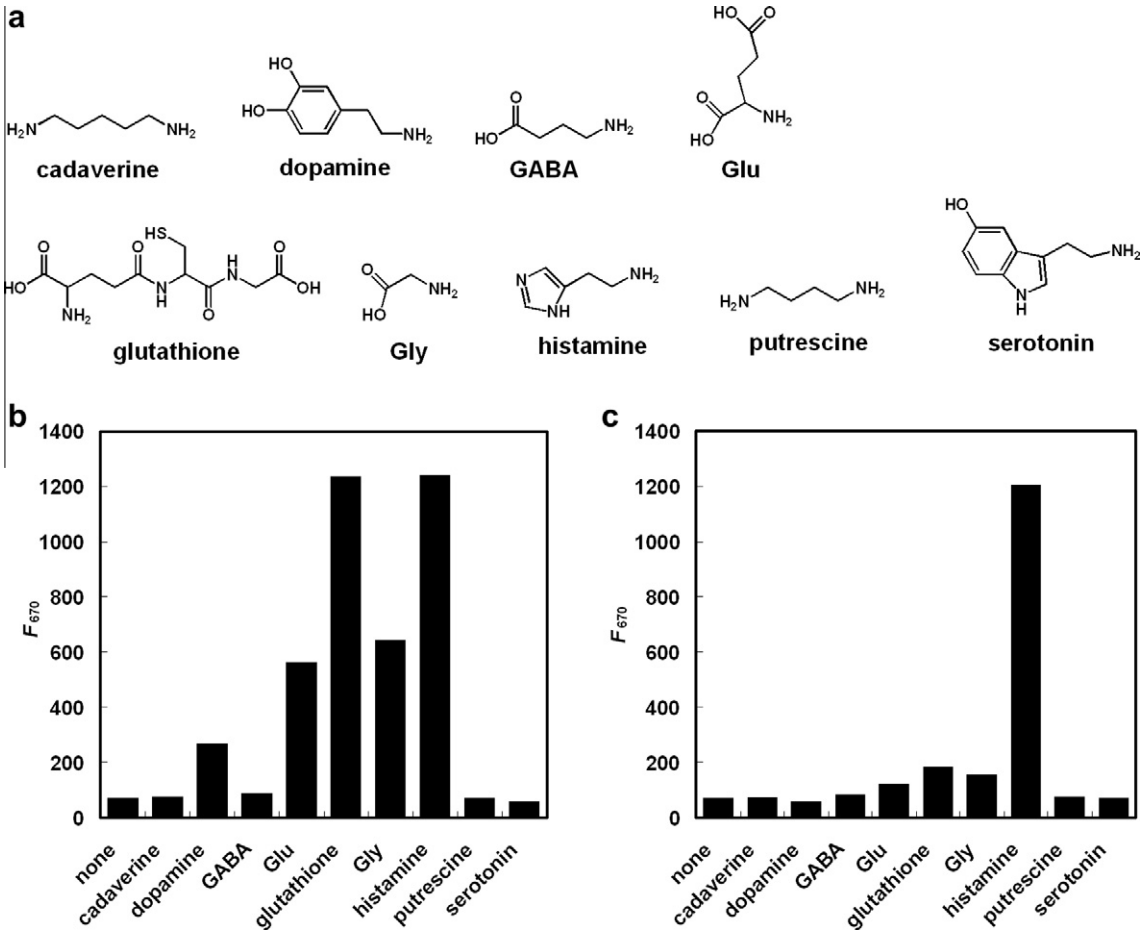

Figure 2. Selectivity of **1**-Cu^{2+} and **1**-Ni^{2+} for histamine. (a) Molecular structures of the biogenic amines tested. (b) Fluorescence responses of **1**-Cu^{2+} (1 μM) to various biogenic amines (2 mM) in D-PBS. (c) Fluorescence responses of **1**-Ni^{2+} (1 μM) to various biogenic amines (2 mM) in D-PBS. The excitation wavelength was set at 600 nm.

histamine may be due to the higher affinity of Cu^{2+} ion for glutathione than other amines. In contrast, **1**-Ni^{2+} showed a high selectivity for histamine and no significant fluorescence response to glutathione and other amines was found, as shown in Figure 2c. The difference in selectivity for the biogenic amines between **1**-Cu^{2+} and **1**-Ni^{2+} can be attributed to the properties of the metal ions. It has been reported that an IDA–Cu^{2+} complex adsorbs proteins via many residues that are stronger than an IDA–Ni^{2+} complex does, while the IDA–Ni^{2+} complex had a higher selectivity for histidyl residues compared to the IDA–Cu^{2+} complex.[16] Fortunately, the above findings would depend on the high selectivity of the IDA–Ni^{2+} complex for histamine. A fluorescent assay based on a ligand exchange reaction, in which the specific and selective fluorescence response to histamine over other amines and a thiol were observed, does not appear to have been reported previously. It is therefore conceivable that **1**-Ni^{2+} could serve as a suitable probe for visualizing histamine in living cells.

Figure 3 shows the time course for the fluorescence response of **1**-Ni^{2+} in the presence of various concentrations of histamine and the relationship between the histamine concentration and fluorescence intensity at a wavelength of 670 nm. The fluorescence intensity of the probe increased immediately on the addition of

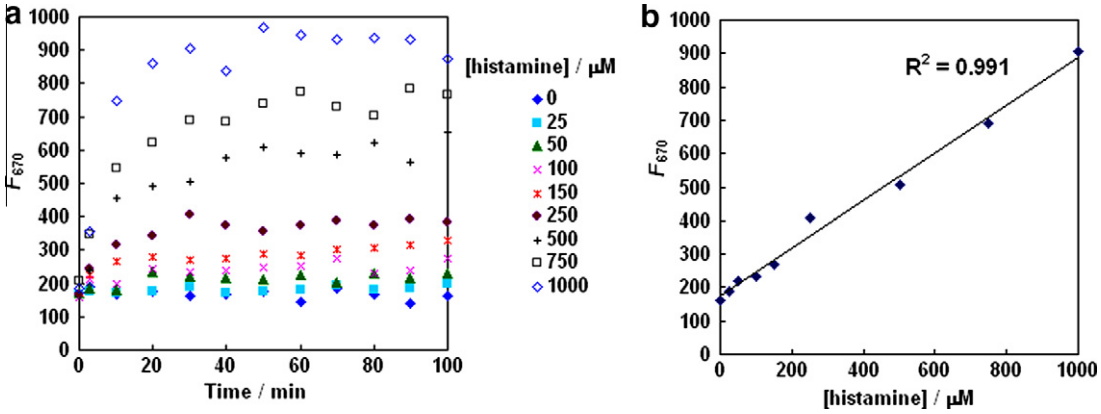

Figure 3. (a) Time course for the fluorescence intensities of **1**-Ni^{2+} (2 μM) upon the addition of histamine (0–1000 μM). The wavelength of excitation was set at 595 nm. (b) Typical standard curve of **1**-Ni^{2+} for histamine. The intensities were obtained at 30 min after the addition of histamine.

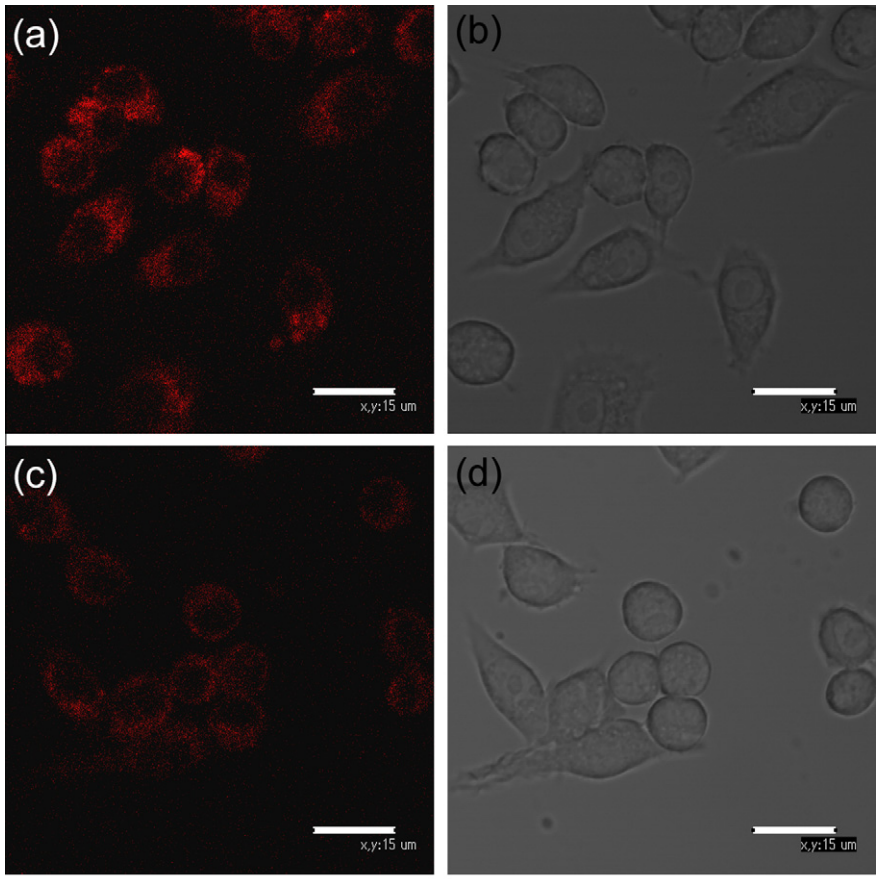

Figure 4. Confocal fluorescence images and bright field images of murine macrophage RAW264 cells. (a and b) Images of RAW264 cells treated with **1**-Ni^{2+} (50 μM) after incubation with histamine (10 mM). (c and d) Images of RAW264 cells treated with **1**-Ni^{2+} (50 μM) without incubation with histamine (control). Scale bar, 15 μm.

histamine and gradually reached a constant value after ca. 30 min. On the other hand, in the case where **1**-Cu^{2+} was used instead of **1**-Ni^{2+} as a probe, the fluorescence intensity was observed to increase rapidly and reach a constant value within 5 min, indicating that **1**-Cu^{2+} responded faster than **1**-Ni^{2+} (data not shown). It is known that the rate of a ligand exchange reaction with water is dependent on the species of metal ion. For example, the rate constant for Cu^{2+} is in the order of 10^{-8} to 10^{-9} s^{-1}, while that for Ni^{2+} is in the order of 10^{-5} to 10^{-6} s^{-1}. Therefore the rate of the fluorescence response of the present probe to histamine can be determined by the ligand exchange rate of the Ni^{2+} ion between histamine and compound **1**. As shown in Figure 3b, the fluorescence intensity increased with increasing histamine concentration and the relationship between the two parameters showed good linearity in the concentration range of 0–1000 μM. The results indicate that **1**-Ni^{2+} would be useful for monitoring histamine in living cells over wide concentration range. In addition, the detection limit was determined to be 54 μM in the present assay.

We then applied **1**-Ni^{2+} to confocal laser scanning microscopy (CLSM) imaging using a macrophage cell line, RAW264. It is known that RAW264 cells actively take up and accumulate histamine, when the concentration of histamine surrounding the cells are higher than a threshold concentration.[17] RAW264 cells were incubated in the absence or presence of 10 mM histamine for 24 h in a 5% CO_2 incubator at 37 °C. The cells were washed with D-PBS, and then treated with 50 μM **1**-Ni^{2+} for 5 min. After the treatment with **1**-Ni^{2+} and washing with D-PBS, CLSM imaging was performed in Opti-MEM medium, to monitor the histamine transported into the cells. As can be seen from Figure 4a, RAW264 cells that had been treated with histamine showed significant fluorescence. However, the fluorescence response for cells that had not been treated with histamine was negligible (Fig. 4c). These findings confirm that this probe is readily transported into living cells and can be used to detect histamine in vivo.

In summary, we report herein the development and testing of a novel fluorescent probe that permits histamine to be detected in living cells based on a ligand exchange mechanism. The probe exhibits water solubility and selectively reacts with histamine. Furthermore, the probe contains a cell membrane permeable dye and has the ability to permit histamine to be directly visualized in living cells. The probe has the potential for use in exploring the neurobiological roles of histamine in living systems, and as a result, it should find widespread use in biological studies.

Acknowledgments

The present work was supported by a Grant-in-Aid for the Global COE Program, 'Science for Future Molecular Systems' from the Ministry of Education, Culture, Science, Sports, and Technology of Japan. D.S. acknowledges the financial support of Grant-in-Aid for JSPS Fellows.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.003.

References and notes

1. (a) András, F.; Merétey, K. *Immunol. Today* **1992**, *13*, 154; (b) Hirasawa, N.; Watanabe, M.; Mue, S.; Tsurufuji, S.; Ohuchi, K. *Inflammation* **1991**, *15*, 117; (c) Hirasawa, N.; Ohuchi, K.; Watanabe, M.; Tsurufuji, S. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 967; (d) Schwartz, J. *Life Sci.* **1979**, *25*, 895.
2. (a) Beaven, M. A.; Rogers, J.; Moore, J. P.; Hesketh, T. R.; Smith, G. A.; Metcalfe, J. C. *J. Biol. Chem.* **1984**, *259*, 7129; (b) Maeyama, K.; Sasaki, M.; Watanabe, T. *Anal. Biochem.* **1991**, *194*, 316.
3. Schwartz, J.; Pollard, H.; Quach, T. T. *J. Neurochem.* **1980**, *35*, 26.
4. (a) Tsuruta, Y.; Kohashi, K.; Ohkura, Y. *J. Chromatogr., B: Biomed. Appl.* **1978**, *146*, 490; (b) Yoshitake, T.; Yamaguchi, M.; Nohta, H.; Ichinose, F.; Yoshida, H.; Yoshitake, S.; Fuxe, K.; Kehr, J. *J. Neurosci. Methods* **2003**, *127*, 11.
5. (a) Niwa, O.; Kurita, R.; Hayashi, K.; Horiuchi, T.; Torimitsu, K.; Maeyama, K.; Tanizawa, K. *Sens. Actuators, B* **2000**, *67*, 43; (b) Bao, L.; Sun, D.; Tachikawa, H.; Davidson, V. L. *Anal. Chem.* **2002**, *74*, 1144.
6. (a) Li, Y.; Kobayashi, M.; Furui, K.; Soh, N.; Nakano, K.; Imato, T. *Anal. Chim. Acta* **2006**, *576*, 77; (b) Lim, T.; Ohta, H.; Matsunaga, T. *Anal. Chem.* **2003**, *75*, 3316.
7. Peng, Q.; He, J.; Jiang, C. *Luminescence* **2009**, *24*, 135.
8. Greenspan, P.; Fowler, S. D. *J. Lipid Res.* **1985**, *26*, 781.
9. Futaki, S.; Ohashi, W.; Suzuki, T.; Niwa, M.; Tanaka, S.; Ueda, K.; Harashima, H.; Sugiura, Y. *Bioconjug. Chem.* **2001**, *12*, 1005.
10. Tsien, R. Y. *Nature* **1981**, *290*, 527.
11. (a) Dean, K. E.; Klein, G.; Renaudet, O.; Reymond, J. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1653; (b) Choi, M. G.; Cha, S.; Lee, H.; Jeon, H. L.; Chang, S. *Chem. Commun.* **2009**, 7390; (c) Chung, S.; Nam, S.; Lim, J.; Park, S.; Yoon, J. *Chem. Commun.* **2009**, 2866; (d) Fu, Y.; Li, H.; Hu, W. *Sens. Actuators, B* **2008**, *131*, 167.
12. Seto, D.; Soh, N.; Nakano, K.; Imato, T. *Anal. Biochem.* **2010**, *404*, 135.
13. Jun, E. J.; Kim, J.; Swamy, K. M. K.; Park, S.; Yoon, J. *Tetrahedron Lett.* **2006**, *47*, 1051.
14. Kamoto, M.; Umezawa, N.; Kato, N.; Higuchi, T. *Chem. Eur. J.* **2008**, *14*, 8004.
15. Sparano, B. A.; Koide, K. *J. Am. Chem. Soc.* **2007**, *129*, 4785.
16. (a) Chaga, G. S. *J. Biochem. Biophys. Methods* **2001**, *49*, 313; (b) Gaberc-Porekar, V.; Menart, V. *J. Biochem. Biophys. Methods* **2001**, *49*, 335; (c) Armisén, P.; Mateo, C.; Cortés, E.; Barredo, J. L.; Salto, F.; Diez, B.; Rodés, L.; García, J. L.; Fernández-Lafuente, R.; Guisán, J. M. *J. Chromatogr., A* **1999**, *848*, 61.
17. Tanaka, S.; Deai, K.; Inagaki, M.; Ichikawa, A. *Am. J. Physiol. Cell Physiol.* **2003**, *285*, 592.