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A novel and simple fluorescence probe for detecting main group magnesium ion in HeLa cells and *Arabidopsis*

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ABSTRACT

A simple-molecule fluorescence probe L has been designed, synthesized and characterized, which shows high selectivity and sensitivity for the main group magnesium ion through fluorescence "turn-on" response in ethanol solution, and no interference from calcium ion in particular. Detection limit of probe L is 1.47×10^{-6} M and the rapid response could reach about 15–20 s. The recognition mechanism has been established by fluorescence spectra, ^{1}H NMR study. Moreover, probe L presents a great photostability, low toxicity and cellular permeability, then we have carried out fluorescent bio-imaging of the probe L for magnesium ions in HeLa cells, which showed that probe L could be utilized to detect the intracellular magnesium ion. Furthermore, it is successfully used as a magnesium ion developer in plant tissues, which shows that it not only can be well tracking the transport of magnesium ion but also make a corresponding fluorescence response to different concentrations magnesium ion. These results would make this probe a great potential application for detecting Mg^{2+} in biological system.

1. Introduction

Magnesium is the most abundant divalent cation within cells, fourth most abundant cation in the body, and the second most common cation in intracellular free fluid. Intracellular Mg^{2+} plays critical roles in many physiological processes including the regulation of hundreds of enzymes (e.g. RNA polymerases, ATPases, protein kinases, phosphatases, glutathione synthase, and carboxylases and so on), activities of membrane proteins including ion channels (Fujii et al., 2014; Komatsu et al., 2004; Politi and Preston, 2003; Gwanyanya et al., 2004). In the human body, Magnesium deficiency can result from a variety of causes including gastrointestinal, renal losses, hypocalcaemia and neurological manifestations, especially, a number of chronic diseases, such as diabetes, osteoporosis, and coronary heart disease have been associated with chronic low magnesium (Jahnen-Dechent and Ketteler., 2012; Fabbrizzi and Poggi., 1995). In plants, Magnesium deficiency which is a widespread problem can affect plant growth and development, which led to the decrease of productivity and quality in agriculture. On the other hand, high levels of Mg^{2+} in the environment will become toxic to plants (Tang et al., 2015), which can enhance the role of antagonism between ions when crops absorb the nutrient elements. Therefore, it is urgent to develop efficient analytical methods for detecting and monitoring Mg^{2+} at the level of cells and tissues in animals and plants.

Over the last few decades, many conventional analytical techniques have been reported for detecting Mg^{2+}, which include atomic absorbance, Mg^{2+}-selective electrodes, null-point titration techniques and NMR (Di Francesco et al., 1998; Rink et al., 1982; Stewart et al., 2016; Flatman and Lew, 1977; Parlak and Turner, 2016). These methods require troublesome sample pretreatment, reagent preparation, time-consuming, and complicated instruments. In addition, some of them are not sensitive enough to determine low concentration of ions. However, with the development of analytical techniques, fluorescence spectroscopy has become a powerful tool for sensing and imaging trace amounts of samples because of its simplicity, sensitivity and fast response times. To date, a number of fluorescent probes of Mg^{2+} have been developed (Haugland, 2005), these probes have receptor groups based upon moieties including diaza-18-crown-6, benzo-15-crown-5, calyx, arene, benzo chromene, imidazo-1,10-phenanthroline and other (Komatsu et al., 2004; Farruggia et al., 2006; Hama et al., 2007; Maguire and Cowan., 2002; Song et al., 2007; Kim et al., 2007). But most of the reported probes suffer from Ca^{2+} interference because of stronger binding affinity for Ca^{2+} than for Mg^{2+}, and until now, only a few small-molecule

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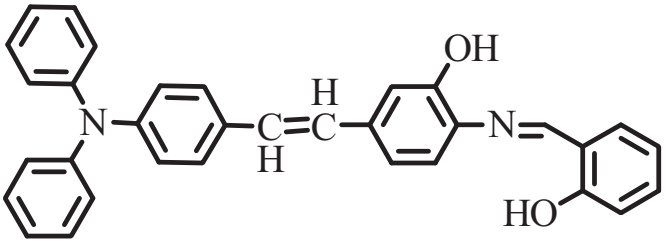

Scheme 1. The structure of probe L.

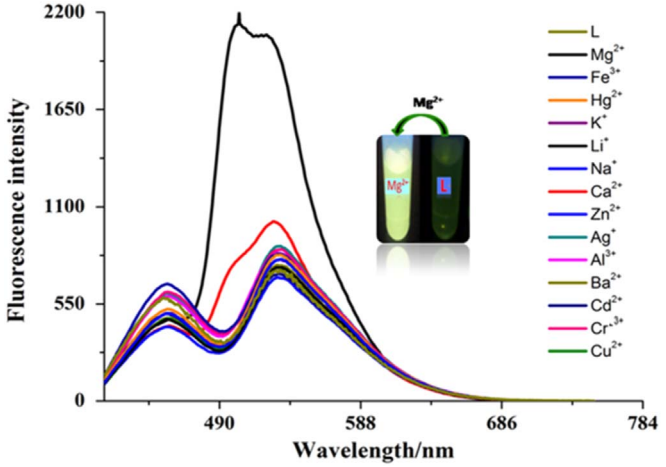

Fig. 1. Fluorescence spectra of probe L (50 μM) in EtOH upon addition of 1equiv. of different nitrate salts (5 μM). Inset: color of L and L+Mg^{2+} under UV lamp at 365 nm.

fluorescent probes for Mg^{2+} have been reported which could simultaneously apply in animal cells and plant tissues.

Inspired by this, we have driven 2-Hydroxybenzaldehyde to react with arylamine to generate o - hydroxyl Schiff base that possessing O and N atoms with specific affinity to Mg^{2+} based on hard and soft acids and bases rule (HSAB) (Joo et al., 2014). We also know that the fluorescence could be quenched when there exist photo induced electron transfer (PET) and C=N double bond isomerization in o-hydroxyl Schiff base (Zhang et al., 2014; Karakaya and Algi, 2014), however, when it coordinates with Mg^{2+}, these processes will be blocked. Hence, we choose o-hydroxyl Schiff base as recognizer to realize "off-on" fluorescence recognition (Scheme 1).

Herein we demonstrated the recognition performance of probe L for Mg^{2+} in ethanol solution, and the simple structure molecule showed the rapid respond ability for $Mg^{2+}$with no interference from calcium ion as we expected. Besides, probe L presented a great photostability, which is the necessary prerequisite for application in fluorescent bio-probes. What's more, probe L could detect the intracellular Mg^{2+} in HeLa cells and Arabidopsis tissues by fluorescence bio-imaging under confocal laser scanning microscopy (CLSM). All of the above results make probe L have a superior potential for analyzing Mg^{2+} in complex bio-systems.

2. Experimental section

2.1. General synthesis of probe L

Intermediate **M** was prepared according to our previous methods (Yang et al., 2013).

L: 2-Hydroxybenzaldehyde (0.097 g, 0.793 mmol) was dissolved in 20 mL ethanol solution and added dropwise into 20 mL ethanol solution of **M** (0.20 g, 0.529 mmol). The mixed solution was stirred at room temperature, and red solid gradually precipitated after 0.5 h. The solid was filtrated after the reaction completed and recrystallized with ethanol to produce 0.21 g solid. Yield: 84.2%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 13.81 (s, 1H), 9.80 (s, 1H), 9.01 (s, 1H), 7.63 (J=8, d, 1H)7.54 (J=8, d, 2H), 7.31–7.42 (m, 6H), 7.04–7.15 (m, 10H), 6.93–6.97 (J=16, t, 4H).^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 160.70, 160.61, 151.44, 146.91, 146.76, 137.33, 133.96, 132.17, 131.18, 129.39, 127.67, 126.42, 124.14, 123.27, 122.89, 119.63, 118.71, 117.95, 116.66, 113.98. FT-IR (KBr, cm^{-1}): 3461.76 (m), 3026.76.50 (m), 2589.44 (w), 1617.68 (s), 1587.23 (s), 1489.76 (s), 1443.13 (m), 1315.64 (m), 1276.78 (s), 1140.38 (s), 752.58 (m), 696.88 (m), 643.61 (m), 525.07(m). MS (ESI): calcd for $[M]^+$: 482.20; found, 483.2070.

2.2. Procedures of the metal ion sensing

Stock solutions (1.0×10^{-2} M) of the nitrate salts of Ca^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Cu^{2+}, Cd^{2+}, Ag^{+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Li^{+}, K^{+}, Na^{+}, Ba^{2+}in aqueous solution and the host probe (1.0×10^{-3} M) of L in DMSO was prepared respectively. Test solution was prepared by placing 50 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each ions stock, and diluting the solution to 5 mL with ethanol respectively. For all measurements, excitation and emission slit widths were both 5 nm.

2.3. cell culture and co-staining

For HeLa cells, the medium used was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum

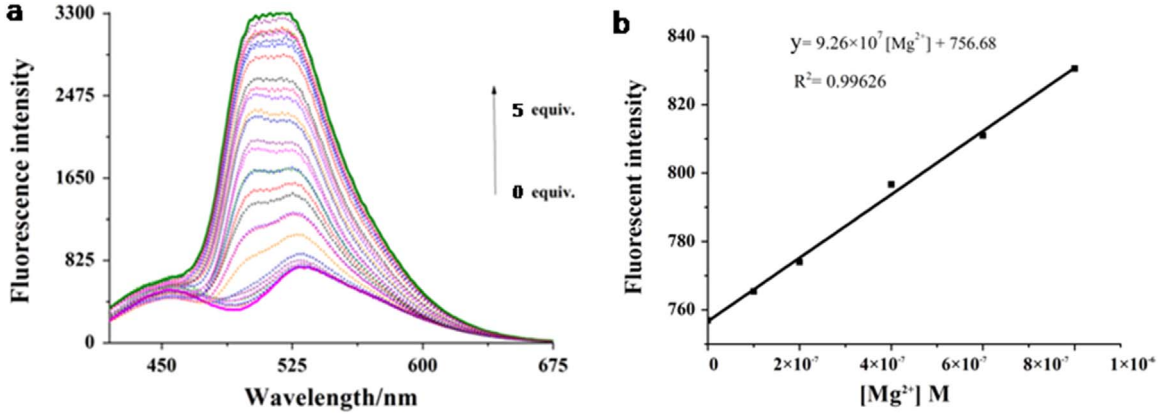

Fig. 2. a Fluorescence titration curves of probe L in EtOH solution (10 μM) upon addition of Mg^{2+} from 0 equiv. to 5 equiv; **b** Calibration curve of L−Mg^{2+} in Ethanol solution.