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A ratiometric fluorescent probe for magnesium employing excited state intramolecular proton transfer

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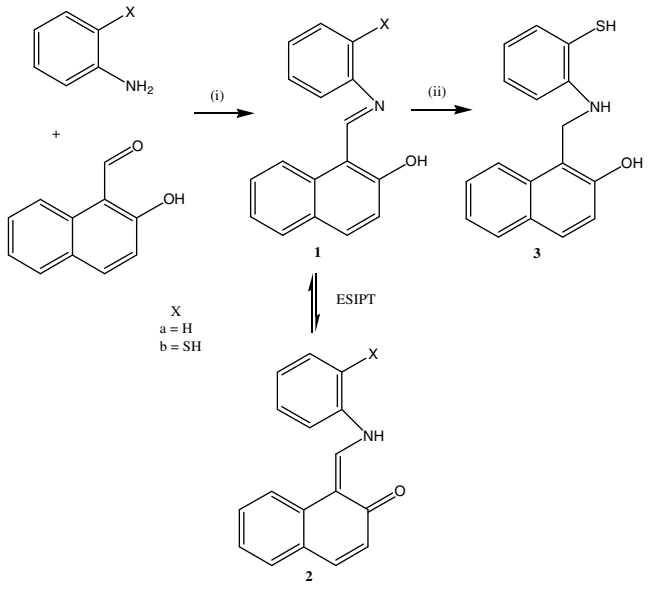
ABSTRACT

A simple fluorescent sensor has been developed for the ratiometric recognition of Mg^{2+} in semi-aqueous solution at pH 7.0. The sensor, a Schiff base, undergoes Excited State Intramolecular Proton Transfer (ESIPT) to generate a keto tautomer with proficient Mg^{2+} binding capability. The sensor displays good selectivity over other metal ions including alkali/alkali earth ions and can measure Mg^{2+} ion concentration between 2.0 and 30.0 μM. The binding stoichiometry was established as 2:1 (host:guest) with an association constant (K_{21}) of $(1.4 \pm 0.1) \times 10^4\ M^{-2}$. The sensor could potentially be used to detect conditions such as hypermagnesaemia.

Magnesium is the second most abundant intracellular cation and the fourth most abundant cation in the body.[1] It plays a vital role as a coenzyme in phosphate transfer reactions, is a cofactor in DNA synthesis and helps modulate signal transduction.[2] Magnesium sulfate is currently used to treat numerous conditions including arrhythmias, myocardial infarction and eclampsia.[3] It is also experiencing a resurgence in its use as a treatment for vasospasm, a complication that is common in patients with subarachnoid haemorrhage (SAH) and which can lead to death due to cerebral ischemia and infarction.[4] SAH is responsible for about 5% of the 110,000 cases of stroke that occur in the UK each year. However, the use of magnesium sulfate as a treatment for vasospasm may be iatrogenic if it leads to hypermagnesaemia.[5] In extreme cases, hypermagnesaemia can result in cardiac arrest and, therefore, simple, rapid methods to detect this condition have obvious benefits.

Efficient detection of magnesium requires a method with high sensitivity, selectivity for Mg^{2+} over Ca^{2+} and an ability to operate in aqueous or semi-aqueous media.[6] Fluorescence spectroscopy continues to play an important role in molecular sensing due to its high sensitivity, rapid response rate and relative inexpense. However, most of the reported fluorescent probes for Mg^{2+} are non-ratiometric, that is, they operate using a single wavelength.[7] Ratiometric fluorescent recognition has advantages over conventional monitoring at a single wavelength as the method is free from the errors associated with receptor concentration, photobleaching and environmental effects.[8] Various strategies have been reported for the development of ratiometric fluorescent sensors.[9] The excited state intramolecular proton transfer (ESIPT) process in sensor **1b** can provide dual channel emission[10] along with the keto tautomer (**2b**) as an efficient receptor for Mg^{2+} (see Scheme 1).[7a–d]



Scheme 1. Reagents and conditions: (i) Dry MeOH, 30 min; (ii) $NaBH_4$, THF/MeOH, 5 h.

The sensor **1b** and control compound **1a** were synthesized by reacting 2-aminothiophenol and aniline, respectively, with 2-hydroxy-1-naphthaldehyde in MeOH following known procedures.[11] Control compound **3** was prepared by reduction of **1b** using $NaBH_4$ in a THF/MeOH solvent system (see Supplementary data).

A 10 μM solution of **1b** in a THF/H_2O (9:1, v/v) solvent system exhibited a fluorescence spectrum with dual emission at λ_{max} = 355 and 427 nm (Fig. 1a) when excited at 275 nm. These two bands

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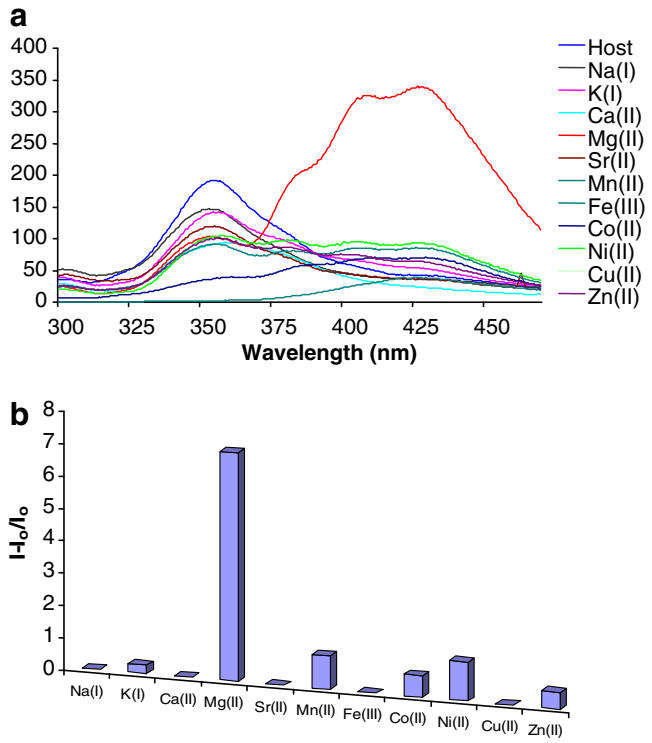

Figure 1. (a) Changes in fluorescent intensity of sensor **1b** (b) fluorescence ratio $(I - I_o/I_o)$ of sensor **1b** upon addition of a particular metal salt in THF/H_2O (9:1,v/v) HEPES buffer solution (pH 7.0 ± 0.1). [**1b**] = 10 μM, [metal salt] = 25 μM.

represent the enol (**1b**) and keto (**2b**) forms, respectively. The cation recognition behaviour of **1b** was evaluated from changes in fluorescence intensity upon addition of a particular metal salt. Upon addition of a 25 μM solution of Mg^{2+} to a solution of **1b**, the intensity of the emission band at λ_{max} = 355 nm decreased along with a concomitant increase of the band at λ_{max} = 427 nm. Other metal ions including alkali (Na^+ and K^+), alkaline earth (Ca^{2+} and Sr^{2+}) and transition metal ions (Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) revealed no such significant change in the fluorescence spectrum under the same conditions. In contrast, control compound **1a**, with the thiol group absent, exhibited no particular binding affinity for Mg^{2+} or any other metal ion except for Cu^{2+} and Fe^{3+} (Fig. S7, Supplementary data).

Similarly, control compound **3**, the reduced Schiff base, was also tested for cation selectivity under the same conditions as used for **1b**. The fluorescence spectrum of receptor **3** did not show any dual emission and no pronounced Mg^{2+} binding affinity (Fig. S8, Supplementary data). This reveals the importance of the imine linkage for the operation of keto–enol tautomerism due to the ESIPT mechanism and also established that the keto form is responsible for the effective binding of Mg^{2+}. The fact that the UV–vis spectra of **1b** before and after the addition of 50 μM Mg^{2+} (Fig. 2) remain relatively unchanged illustrates that **1b** does not bind Mg^{2+} in the ground state and that excitation is required to produce the Mg^{2+} chelating keto tautomer. The binding pattern of **1a** revealed the significance of the thiol group in Mg^{2+} binding. Though the thiol group is usually considered a soft binding site and is generally expected to play a minor role in binding alkali/alkali earth metal ions such as Mg^{2+}, the results from the analysis of **1a** show its inclusion is mandatory for the efficient binding of Mg^{2+} by **1b**. We further confirmed this by oxidizing a sample of **1b** to its disulfide analogue which destroyed the affinity for Mg^{2+}. Therefore, it appears that the core functionality required for **1b** to efficiently bind Mg^{2+} upon excitation are a thiol group, an imine linkage and a hydroxy group.

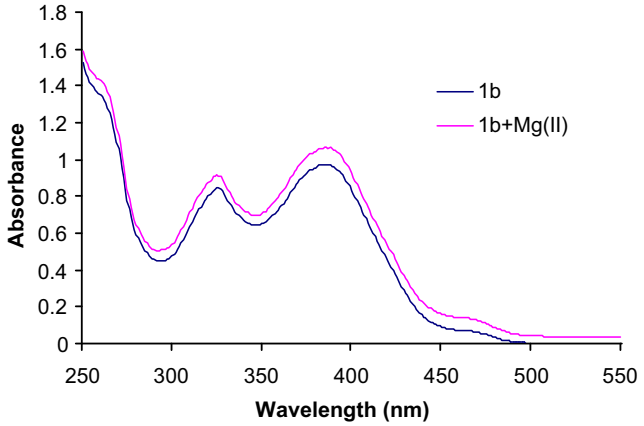

Figure 2. UV-Vis spectra of receptor **1b** and **1b** in the presence of Mg^{2+}. [**1b**] = 25 μM, [Mg^{2+}] = 50 μM.

We also investigated the response of **1b** to variations in solution pH (Fig. S9, Supplementary data). At low pH, the fluorescence intensity at λ_{max} = 355 nm, increases, most likely due to the inhibition of photoinduced electron transfer (PET) from the lone pair of the sp^2 nitrogen to the fluorophore. This protonation removes the possibility of ESIPT and, therefore, no changes were observed at 427 nm. Interestingly, high pH also switched on the fluorescence of **1b** but at λ_{max} = 345 nm. We believe this is due to deprotonation of the phenolic hydroxyl group which otherwise causes non-radiative decay from the excited state by vibrationally coupling the excited state to water.[12] This deprotonation also removes the possibility of ESIPT and so no major changes were observed at 427 nm. However, at intermediate pH (pH 7.0) fluorescence intensity remained low. Therefore, by working in HEPES buffered solution we ensure the phenolic group in **1b** remains protonated and can engage in effective ESIPT.

Figure 3a shows the changes in the fluorescence spectra of **1b** upon titration of Mg^{2+} ions. The results show that upon the continuous addition of Mg^{2+} ions to a solution of **1b** in THF/H_2O (9:1,v/v), the intensity of the emission band decreased at λ_{max} = 355 nm, and the intensity of the band at λ_{max} = 427 nm started to increase rapidly. We believe the reason for this to be as follows: in the absence of Mg^{2+} the enol form is in equilibrium with its keto tautomer in the excited state. As Mg^{2+} binds to the keto form this is removed from the equilibrium which adjusts by producing more keto tautomer. Thus, the intensity due to the enol tautomer decreases while that from the Mg^{2+}-bound keto tautomer increases. Therefore, **1b** can be used for selective ratiometric estimation of Mg^{2+} along a concentration range of 2.0–30.0 μM (Fig. S10, Supplementary data).[13] To determine the ability of **1b** to measure Mg^{2+} ions in the presence of other physiologically important ions, a competitive binding experiment was performed. Figure 3b represents the comparison of the ratiometric fluorescence intensity upon Mg^{2+} binding with and without a Ca^{2+} background. The results show that the estimation of Mg^{2+} is free from any error due to an equimolar concentration of Ca^{2+}.

To determine the binding stoichiometry between receptor **1b** and Mg^{2+}, the continuous variation method was used.[14] Figure 4 shows the Job plot of the fluorescence intensity of free **1b** and the intensity of the system with the molar fraction of the host {[H]/([H] + [G])} for a series of solutions, in which the total concentration of host and guest was constant, with the molar fraction of host continuously varying. The results indicate the formation of a 2:1 (Host:Guest) complex. Using the equation: $[G]_{tot} = a/2K_{21}(1 - a)^2[H]_{tot} + a[H]_{tot}/2$, where $[G]_{tot}$ is total concentration of guest, $[H]_{tot}$ is the total concentration of host, $a = (I - I_o)/(I_i - I_o)$ with I

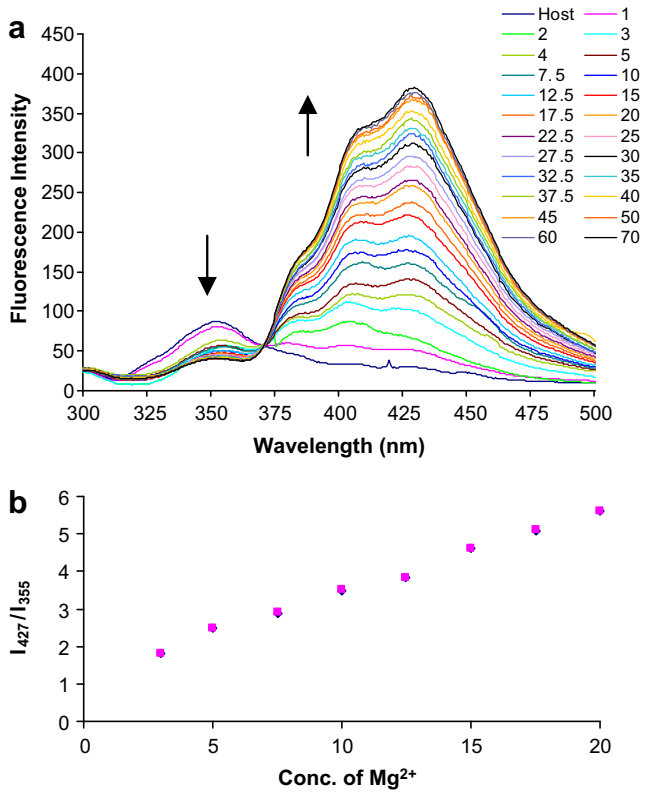

Figure 3. (a) Changes in fluorescent spectra of receptor **1b** (10 μM) upon successive additions of Mg^{2+} (0–70 μM) in THF/H_2O (9:1,v/v) HEPES buffer solution (pH 7.0 ± 0.1). (b) Plot of ratiometric fluorescence intensity (I_{427}/I_{355}) of **1b** against metal ion concentration for Mg^{2+} (μM) (♦); for Mg^{2+} in the presence of equimolar Ca^{2+} (μM) (■).

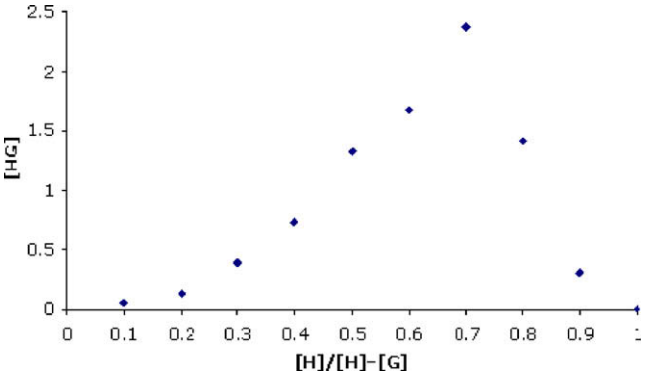

Figure 4. Job plot showing the 2:1 (H:G) stoichiometry of a complex formed between receptor **1b** and Mg^{2+}.

being the fluorescent intensity at a particular Mg^{2+} concentration while I_o and I_i are the intensities at zero and infinite Mg^{2+} concentrations, respectively, the association constant K_{21} was determined as $1.4 \times 10^4 \pm 0.1\ M^{-2}$.[15] This value is lower than expected from non-linear curve fitting and may be explained as follows: Mg^{2+} binding results in the association of two receptors that were previously free in solution causing an unfavourable change in the entropy of the system.[16] In addition, Mg^{2+} must compete with the keto–enol tautomerization process before it can form a complex with the keto form. These conflicting processes result in a lower association constant than might be expected.

In summary, we have developed a novel fluorescent sensor for the ratiometric determination of Mg^{2+}. The formation of a keto tautomer by ESIPT appears to be critical for Mg^{2+} recognition and also provides dual emission that enables ratiometry. To the best of our knowledge this is the first reported example of a ratiometric probe for magnesium that utilizes ESIPT. The probe does not suffer from interference by Ca^{2+} and may provide a simple, rapid detection method for hypermagnesaemia.

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Supplementary data

Supplementary data (available including experimental details, spectra and graphs) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.09.052.

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