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A highly selective and sensitive turn-on fluorescence chemosensor based on a rhodamine–adenine conjugate for Al^{3+} in aqueous medium: Bioimaging and DFT studies

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ABSTRACT

Recently, extraordinary progress has been made for the specific detection of environmentally and biologically relevant metal ions in aqueous solution and living cells. A new dye **L** where rhodamine B is covalently attached to adenine has been synthesized in good yields. This dye is found to be highly specific in detecting of Al^{3+} ion in presence of excess of biologically relevant metal ions in DMSO/H_2O [1:4 (v/v), HEPES buffer, pH=7.1] medium. Upon complexation with Al^{3+} the dye exhibits a substantially enhanced absorbance intensity at 566 nm and fluorescence intensity at 590 nm. Besides showing high selectivity, the sensor gives a sensitivity of 0.19 μM for Al^{3+} in both absorbance and fluorescence studies over a wide pH range. The sensitivity is much higher compared to the value mentioned in the guidelines of WHO (7.41 μM). Moreover, the dye shows moderately cytotoxicity and can be employed for the detection of intracellular concentration of Al^{3+} ions in living cells.

1. Introduction

The development of efficient chemosensors and chemodosimeters for various cations and anions has received strong attention because of the sensitivity of the techniques pertaining to environmental and biological aspects [1–4]. Aluminum is the third most abundant element (approximately 8% of total mineral components) behind oxygen and silicon in the earth crust and its concentration in water are much bigger than biologically relevant cations such as Mg(II), Fe(III) or Zn(II) [5,6]. Some 40% of the world's soil acidification occurs due to increased solubility of aluminum minerals through acid rain [7,8]. Presently, aluminum is widely used in water treatment, as a food additive and as aluminum based pharmaceuticals. Besides, this metal can get into biosystems as occupational dusts from industrial activities such as manufacturing of cars, computers, aluminum containers and cooking utensils [9]. The average daily human intake of aluminum is approximately 3–10 mg per day [10,11]. But even a slightly more chronic exposure of the ion may cause Parkinson's disease, Alzheimer's disease and damage to the central nervous system in human [12,13]. It also impairs the functioning of iron–sulfur proteins by reducing ferritin levels in all tissues [14]. Because of its similar atomic size and electric charge (0.051 nm and 3+, respectively), aluminum ion can substitute essential metal ions such as magnesium (0.066 nm, 2+), calcium (0.099 nm, 2+), and iron (0.064 nm, 3+) from the active sites of metallobiomolecules with grave consequences. Therefore, the quantitative sensing of Al^{3+} in bio-systems is of great utility in reducing the direct impact of aluminum on human health [15–29].

Among the various detection techniques, fluorescence chemosensors are the most promising methods because of their high selectivity, sensitivity, short response time, simplicity, low detection limits and useful applications in environmental chemistry, medicine and biology [30–32]. Among various fluorophores, the rhodamine framework is a convenient mode to construct fluorescence chemosensors by virtue of its excellent photophysical properties such as large molar extinction coefficient (ε), high fluorescence quantum yield (ϕ) and visible absorption and emission wavelengths. Besides, the rhodamine framework shows fluorescence OFF–ON behavior via switching between the spirocyclic form (which is colorless and non-fluorescent) and the ring-opened amide form (which is pink and strongly fluorescent) and hence, can be used as a chemodosimeter as well.

Metal–nucleic acid interactions have been a subject of considerable interest because of the diverse roles these biomolecules play in living systems. However, it is not certain in most cases whether a metal ion prefers to bind at the phosphate or at the purine/pyrimidine bases [33,34]. While a large volume of studies

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are available relating to interactions of alkali [35,36], alkaline earth [37] or divalent transition metal ions [38–40] with nucleic acid bases, much less is known on the interactions of these bases with trivalent metal ions such as Al^{3+} [41,42].

The sensor **L** is designed to act as a reporter of interactions between adenine and Al^{3+} ion through fluorescence and absorbance studies. In this paper, we report a rhodamine derived adenine "turn-on" fluorescent sensor **L** that is highly specific for Al^{3+} ion in aqueous DMSO medium at physiological pH. The optical properties were investigated using UV–vis and fluorescence response of the dye for Al^{3+} and the bio-imaging application is also reported.

2. Experimental section

2.1. Materials and reagents

Reagent grade rhodamine B, adenine, triethylamine, $POCl_3$ and sodium salts of different anions were bought from S.D. Fine Chemicals (India). All metal perchlorate, nitrate and chloride salts were bought from Sigma Aldrich Chemicals (USA). These chemicals were used as received without further purification. All the solvents were procured from S.D. Fine Chemicals (India) and were purified prior to use following standard procedures. Chromatographic separations were done by column chromatography using basic Al_2O_3 procured from S.D. Fine Chemicals (India). All experimental solutions of varying pH were made with HEPES buffer. The variation of pH was done with dilute HCl and NaOH.

2.2. Instruments

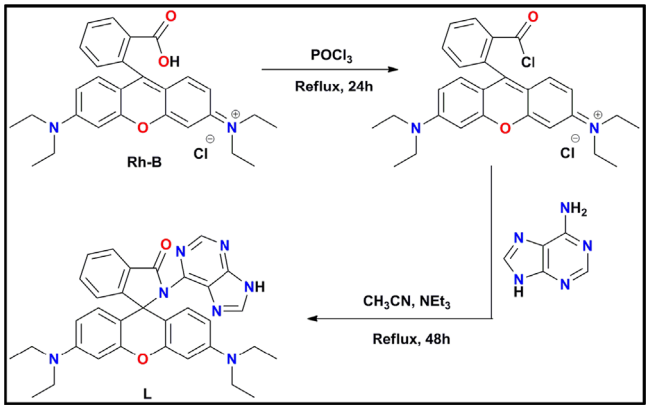
All the compounds were characterized by various spectroscopic methods. Both ^{1}H NMR (500 MHz) and ^{13}C NMR spectra (125 MHz) of the compounds were recorded on a JEOL DELTA2 spectrometer in $CDCl_3$ and DMSO-d_6 with $Si(CH_3)_4$ as the internal standard. The ESI–MS data were obtained in methanol from a WATERS–Q–Tof Premier Mass Spectrometer. UV–vis spectra were recorded on a Shimadzu 2450 UV–vis spectrophotometer in DMSO:H_2O (1:4, v/v) medium at 19 °C. Fluorescence emission spectra were obtained using a Perkin-Elmer LS 50B Luminescence Spectrometer at 19 °C.The imaging system was comprised of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50 watt lamp. The pH of different solutions was measured by using Eco testr pH 1 pH meter.

2.3. Quantum yield calculation

Fluorescence quantum yields (Φ) were estimated by integrating the fluorescence spectrum with that of rhodamine B (Φ=0.49) in ethanol taking the area under the total emission following the equation:

$$\Phi_{sample} = \Phi_{ref}\left(F_S A_R / F_R A_S\right) \times \left(\eta_S/\eta_R\right)^2$$

where Φ stands for quantum yield, F stands for area under the fluorescence spectra, A stands for absorbance value and η stands for the refractive index value of the solvent used. The subscript "R" indicates the value of the parameter for reference (i.e. rhodamine B) and "S" subscript indicates the value of the parameter for the sample. In case of L the quantum yield was determined in absence and presence of 10 equivalent Al^{3+} ions.



Scheme 1. Schematic representation of synthesis of the probe **L**.

2.4. UV–vis and fluorescence spectroscopic studies

The luminescence properties of **L** was checked in a DMSO:H_2O (1:4, v/v), 10 mM HEPES buffer, (pH= 7.1). Stock solution of **L** was prepared at the concentration of 10^{-3} M in 25 mL of DMSO and then diluted to a desired concentration at 19 °C. Stock solutions of various ions were prepared at the concentration of 10^{-3} M in 25 mL in distilled water and then diluted to a desired concentration. In titration experiments, samples were prepared by adding appropriate amounts of the salt stock solution to 2 mL of a solution of **L** in a quartz optical cell of 1 cm optical path length. Fluorescence and absorbance spectral data were recorded at 1 min after the addition of the ions. For fluorescence measurements, excitation was provided at 480 nm (slit width=10/10 nm) and emission was acquired from 540 nm to 750 nm. The study of effect of pH was carried out in 10 mM HEPES buffer solution by adjusting the pH using HCl or NaOH.

2.5. Synthesis of the sensor *L*

Synthesis of the dye **L** was straightforward as illustrated in Scheme 1. Rhodamine B (5 g, 10.44 mmol) was taken in a 250 mL RB flask containing 100 mL freshly distilled $POCl_3$ and allowed to reflux for 24 h under a dinitrogen blanket. The excess amount of $POCl_3$ was removed in a rotary evaporator that provided the corresponding acid chloride. This was dried under vacuum and used for the next step without further purification. The acid chloride was dissolved in dry acetonitrile (100 mL) then adenine (5.64 g, 41.76 mmol) followed by triethylamine (5 mL) were added slowly. Once the addition was complete, the resulting mixture was heated to reflux for 48 h. The reaction mixture was then concentrated in a rotary evaporator under low pressure and the extracted with dichloromethane. The organic layer was after drying over anhydrous Na_2SO_4 was evaporated completely. The residue was purified by column chromatography with the eluent ethylacetate:dichloromethane (20:80, v/v) to afford the product. The product **L** was recrystallized from ethanol as a pale pink solid (1 g, Yield ~16%, m.pt. 237 °C).^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS). δ: 1.00 (t, 12H, J=6.9 Hz), 3.23 (q, 8H, J=6.85 Hz), 6.15 (d, 2H, J=6.9 Hz), 6.30 (s, 2H), 6.45 (d, 2H, J=8 Hz), 7.06 (d, 1H, J=8.05 Hz), 7.62 (m, 2H), 7.99 (d, 1H, J=7.45 Hz), 8.29 (s, 1H), 8.44 (s, 1H) (Fig. S2). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ: 12.70, 44.36, 68.55, 97.57, 106.93, 107.19, 114.40, 123.50, 125.07, 127.46, 128.77, 135.13, 142.00, 142.47, 148.85, 151.42, 154.22, 154.61, 161.61, 169.58 (Fig. S3). ESI–MS: m/z (%): 560.2776 (100%) $[\mathbf{L}+H]^+$ (Fig. S5). Elemental analysis: calcd (%) for $C_{33}H_{33}N_7O_2$: C 70.86, H 5.94, N 17.53; found: C 70.75, H 6.06, N 17.48.