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A new chemodosimetric probe for the selective detection of trivalent cations in aqueous medium and live cells

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ABSTRACT

A new quinoline functionalized Schiff base (**L**) was synthesized which displayed selective fluorescence response to trivalent metal cations (Al^{3+}, Fe^{3+} and Cr^{3+}) over a variety of divalent and monovalent metal cations in ~100% aqueous medium. The specific sensing of trivalent cations by a switch-ON fluorescence response is based on selective acid catalyzed imine bond hydrolysis of **L** facilitated by the higher valence metal ions in aqueous medium and this mechanism was corroborated by mass spectrometry and density functional theory (DFT) calculations. The developed probe was biocompatible and rendered sensing of trivalent metal ions (Al^{3+}, Fe^{3+} and Cr^{3+}) in live HeLa cells through imaging.

1. Introduction

Trivalent metal ions hold significant environmental and biological relevance [1]. For example, aluminium, the third most abundant metal in the Earth's crust, is a very common species of metal cation, which causes drinking water contamination and can be toxic to humans in excessive amounts. Due to acid rain, the concentration of Al^{3+} increases in soil, leading to toxicity in plants [2–4]. Al^{3+} toxicity is implicated in microcytic hypochromic anemia, encephalopathy, myopathy, Al-related bone disease (ARBD) and neuronal disorder leading to dementia and Alzheimer's disease [5,6]. Cr^{3+} is an essential element in human nutrition and can impact metabolism of carbohydrates, fats, proteins and nucleic acids as it activates certain enzymes and stabilizes proteins and nucleic acid. Overdose of Cr^{3+} may lead to disturbance in glucose levels and lipid metabolism [7,8]. A deficiency of Cr^{3+} in humans may cause diabetes and cardiovascular disease [9]. Furthermore, environmental pollution caused by the industrial run-offs of $Cr^{3+,}$ is a matter of grave concern in agriculture [10,11]. Fe^{3+} is the most abundant transition metal in cellular system and has a pivotal physiological significance due to its presence in numerous enzymes and proteins. Iron dyshomeostasis (either deficiency or overload) may cause certain cancers and dysfunction of various organs [12–17]. Iron having adequate redox potential and high affinity for oxygen is critical in both electron transfer reactions and oxygen transport. A number of examples illustrating the role of Fe^{3+} in a variety of cell functions has been reported [18,19].

As compared to the large number of chemosensors developed for divalent transition metal ions there are only a few reported organic probes for recognition of trivalent metal cations [20–22]. Although selective fluorogenic probes for Fe^{3+}, Al^{3+} and Cr^{3+} have been reported [8,23–31], detection of all trivalent metal cations (Al^{3+}, Cr^{3+} and Fe^{3+}) by a single chemosensor is still lacking. Fluorescence-based techniques are attractive for sensing of biological and environmentally relevant metal ions owing to fast, facile and highly sensitive detection of target analytes [32–38]. Generally fluorophores like rhodamine [39–41], fluorescein [42], and BODIPY [43,44] have been used to develop luminescence turn-ON and/or ratiometric sensors. Depending on the structure of the fluorophore and the condition in which the fluorophore is used the sensing mechanism may vary. For example, Barba-Bon et al. reported a highly efficient fluorescent probe for trivalent cations (Al^{3+}, Cr^{3+} and Fe^{3+}), where the fluorescence turn-ON was due to the formation of 1:1 or 2:1 ligand–metal complexes [42]. Recently, Chen et al. developed a pyridinyl-functionalized tetra-phenylethylene probe for the detection of trivalent cations (Al^{3+}, Cr^{3+} and Fe^{3+}) where the fluorescence turn-ON response was explained by the protonation of the organic probe, originating from the higher hydrolyzing ability of M^{3+} over M^{+} and M^{2+} in the water–ethanol mixture [45]. More recently we also have reported a new imidazole functionalized anthracene based Schiff base which revealed

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selective colorimetric and protonation induced turn-ON fluorescence response to trivalent metal cations (Al^{3+}, $Fe^{3+}$and Cr^{3+}) in water–methanol mixture [46].

In our continuous pursuit to design sensors for various analytes [46–50], here in we report a new quinoline functionalized thiophenol based Schiff base (**L**) (Scheme 1), which displays selective and a remarkable turn-ON fluorescence response in presence of trivalent metal cations Al^{3+}, Cr^{3+} and Fe^{3+} over various monovalent and divalent metal ions in ~100% aqueous medium. The selective fluorescent response is essentially based on higher valence metal ion (M^{3+}) assisted acid catalyzed hydrolytic cleavage of imine linkage of the probe (**L**) in the aqueous medium. Sensing of the trivalent metal ions in live HeLa (human cervical carcinoma cells) cells through imaging is also demonstrated in the study.

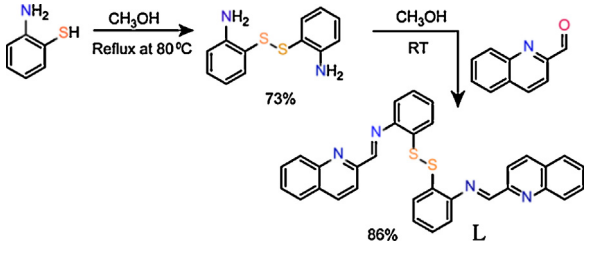
2. Experimental

2.1. General information and materials

All the materials for synthesis were purchased from commercial suppliers and used without further purification. The absorption spectra were recorded on a PerkinElmer Lamda-25 UV–vis spectrophotometer using 10 mm path length quartz cuvettes in the range of 250–800 nm wavelength, while fluorescence measurements were performed on a Horiba Fluoromax-4 spectrofluorometer using 10 mm path length quartz cuvettes with a slit width of 3 nm at 298 K. The mass spectrum of the ligand **L** was obtained using Waters Q-ToF Premier mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT-400 MHz instrument. The chemical shifts were recorded in parts per million (ppm) on the scale. The following abbreviations are used to describe spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.

2.2. Synthesis of *L*

In 30 ml methanol, ~10 mmol (1.1 ml) of 2-aminothiophenol was added and it was refluxed at 80 °C for 5 h. Subsequently the solvent was allowed to evaporate slowly and needle-shape crystals of the corresponding di-amine with S—S bond were obtained (Scheme 1). In the following step ~1 mmol (0.25 g) of the di-amine was added to methanol solution containing ~2 mmol (0.32 g) of 2-quinolinecarboxaldehyde and the mixture was stirred for 12 h to obtain a pale yellow–white colored solid product of **L**. The product was filtered, washed thoroughly with methanol and then dried in the desiccator. The calculated yield of **L** was found to be 86%. ^{1}H NMR [400 MHz, $CDCl_3$, TMS, J (Hz), δ (ppm)]: 8.85 (2H, s), 8.51 (2H, d, J = 8.4), 8.26 (2H, d, J = 8.4), 8.19 (2H, d, J = 8.4), 7.90 (2H, d, J = 7.6), 7.81 (2H, t, J = 8.0), 7.73–7.71 (4H, m), 7.65 (2H, t, J = 8.0), 7.27–7.23 (4H, m), 7.26(1H, s, $CDCl_3$). ^{13}C NMR [100 MHz, $CDCl_3$, TMS, δ (ppm)]: 160.46, 154.95, 148.18, 147.71, 137.00, 133.35, 130.22, 129.93, 129.27, 128.34, 128.12, 128.06, 127.33, 126.20, 119.27, 117.43. (Electrospray ionisation mass spectrometry) ESI-MS (positive mode, m/z) calculated for $C_{32}H_{22}N_4S_2$: 527.1386. Found: 527.1387 [(M+H$^+$)].



Scheme 1. Synthesis of ligand **L**.

2.3. UV–vis and fluorescence spectroscopic studies

Stock solutions of various ions (1×10^{-3} mol l^{-1}) were prepared in deionized water. A stock solution of **L** (1×10^{-3} mol l^{-1}) was prepared in dimethyl sulfoxide (DMSO). The solution of **L** was then diluted to 10×10^{-6} mol l^{-1} with deionized water. In titration experiments, a quartz optical cell of 1 cm path length was filled with a 2.0 ml solution of **L** to which the ion stock solutions were gradually added using a micropipette. Spectral data were recorded within 1 min after addition of the ions. In selectivity experiments, the test samples were prepared by placing appropriate amounts of the cations stock into 2.0 ml of **L** solution (1.0×10^{-5} mol l^{-1}). For fluorescence measurements, excitation was provided at 296 nm, and emission was acquired from 310 nm to 600 nm.

2.4. Calculation of detection limit

The detection limits were calculated on the basis of the fluorescence titrations. The fluorescence emission spectrum of **L** was measured 10 times, and the standard deviation of blank measurement was achieved. To gain the slope, the fluorescence emission at 413 nm was plotted as a concentration of M^{3+} (M^{3+} = Al^{3+}, Cr^{3+} and Fe^{3+}). The detection limits were calculated using the following equation:

$$\text{Detection limit} = \frac{3\sigma}{k} \quad (1)$$

where σ is the standard deviation of blank measurement, and k is the slope between the fluorescence emission intensity versus [M^{3+}].

2.5. Cytotoxic effect on HeLa cells

The cytotoxic effect exerted by ligand and various ligand–metal complexes on cultured HeLa cells (human cervical carcinoma cells) was determined by the well-established MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as per the manufacturer instruction (Sigma–Aldrich, MO, USA). HeLa cells were propagated in a 25 cm^2 tissue culture flask in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% (v/v) FBS (Fetal Bovine Serum), penicillin (100 μg/ml) and streptomycin (100 μg/ml) in a CO_2 incubator until the cells were approximately 90% confluent. Prior to MTT assay, HeLa cells were seeded into 96-well tissue culture plates at a cell-density of 10^4 cells per well and incubated in separate sets with varying concentrations of the ligand, ensemble of ligand and trivalent metal salts and trivalent metal salts alone (all made in DMEM) and incubated for a period of 24 h under 5% CO_2. Solvent control samples (cells incubated in DMSO) were also set up in parallel. Following incubation, the growth media was carefully removed, and fresh DMEM containing MTT solution was added to the wells. Following incubation for 3–4 h at 37 °C, the supernatant was collected and the insoluble colored formazan product was solubilized in DMSO and its absorbance was measured in a microtiter plate reader (Infinite M200, TECAN, Switzerland) at 550 nm. For every concentration of ligand, ligand–metal ensembles and trivalent metal salts, MTT assay was performed in six sets. Data analysis and determination of standard deviation were performed with Microsoft Excel 2010 (Microsoft Corporation). In the MTT assay, the absorbance for the solvent control cells was considered as 100% cell viability and the absorbance for the treated cells was compared to that for the solvent control cells in order to ascertain % cell viability.