Sensors and Actuators B 194 (2014) 120–126

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A new fluorogenic probe for solution and intra-cellular sensing of trivalent cations in model human cells

Soham Samanta[a,1], Sudeep Goswami[b,2], Aiyagari Ramesh[b,**], Gopal Das[a,*]

[a] Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati 781039, India
[b] Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, India

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 7 December 2013
Accepted 10 December 2013
Available online 27 December 2013

Keywords:
Trivalent ion sensor
Fluorescence turn-on
Proton-induced fluorescence change
Cell imaging

ABSTRACT

A new imidazole functionalized anthracene based Schiff base (**L**) was synthesized and it's selective colorimetric as well as fluorescence responses to trivalent metal cations (Al^{3+}, Fe^{3+} and Cr^{3+}) over a variety of divalent and monovalent metal cations in 1:1 methanol–water mixed solvent and in live HeLa cells were demonstrated. These selective colorimetric and fluorescence responses were explained as a consequence of protonation of **L**, where the lower K_{sp} values of M^{3+} (Al^{3+}, Fe^{3+} and Cr^{3+}) facilitated the generation of H^+ through hydrolysis. This fluorogenic behavior of the ligand **L** was further confirmed by theoretical calculations.

1. Introduction

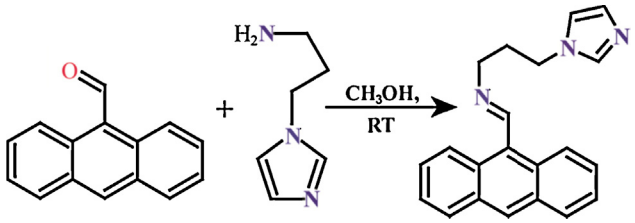
Fluorescent chemosensors of trivalent metal cations (M^{3+}) are very rare and thus it becomes an important research subject. However, trivalent metal cations have a huge impact on research field due to their own biological significance [1] and environmental importance. For example, Cr^{3+}, an essential element in human nutrition, has a huge impact on the metabolism of carbohydrates, fats, proteins and nucleic acids as it activates certain enzymes and stabilizes proteins and nucleic acid. Overdose of Cr^{3+} not only affects negatively cellular structure and function, but also causes disturbance in glucose levels and lipid metabolism [2,3] while a deficiency of Cr^{3+} in humans may cause diabetes and cardiovascular disease [4]. Also, the industrial surplus of Cr^{3+}, causing environmental pollution is a matter of great concern in agriculture [5,6]. Fe^{3+}, the most abundant transition metal in cellular system, has an outstanding biological importance due to its presence in numerous enzymes and proteins. Iron, having adequate redox potentials and high affinity for oxygen can be critically involved in both electron transfer reactions and oxygen transport. A number of examples with important roles of Fe^{3+} in a variety of cell functions have been summarized in tutorial reviews [7,8]. Aluminum is not only the third most abundant metal in the Earth's crust, but also Al^{3+} is very common species of metal cations, 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, which is deadly to living plants [9–11]. Al^{3+} toxicity causes microcytic hypochromic anemia, encephalopathy, myopathy, Al-related bone disease (ARBD), neuronal disorder leading to dementia and Alzheimer's disease [12,13].

Even though a large number of chemosensors for divalent transition metal ions are developed, there are only few reported organic probe for recognition of trivalent metal cations till today [14–16]. Going through several literatures we found that some Fe^{3+} [17–19], Al^{3+} [20–23] and Cr^{3+} [3,24,25] selective fluorogenic probes already have been reported yet, the lack of exploring chemosensors for the detection of all trivalent metal cations (Al^{3+}, Cr^{3+} and Fe^{3+}) by a single fluorogenic probe are quite astonishing. So the development of chemical sensor for the detection of trivalent metal cations in environmental and biological samples is of a genuine need.

In the sense of application, biologically and environmentally relevant metal ions sensing by fluorescent techniques are attractive due to the feasibility of fast, facile and highly sensitive detection of target analytes [26–32]. Generally the fluorogens such as rhodamine [33–35], fluorescein [36], and BODIPY [37,38] have been used to develop luminescence turn-on and/or ratiometric sensors. Depending on the structure of the fluorophore and the situation in which the fluorophore is used the sensing mechanism varies in different cases. For example, Barba-Bon et al. reported a highly efficient fluorescent probe for trivalent cations

* Corresponding author. Tel.: +91 361 2582313; fax: +91 361 2582349.
** Corresponding author. Tel.: +91 361 2582205; fax: +91 361 2582249.
E-mail addresses: aramesh@iitg.ernet.in (A. Ramesh), gdas@iitg.ernet.in, g.das@mailcity.com (G. Das).
[1] Tel.: +91 361 2582313; fax: +91 361 2582349.
[2] Tel.: +91 361 2582205; fax: +91 361 2582249.

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.12.049



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

(Al^{3+}, Cr^{3+} and Fe^{3+}), where the fluorescence turn-on was realized *via* the formation of 1:1 or 2:1 ligand–metal complexes [36]. More recently, Tang et al. has 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, attributed from the higher hydrolyzing ability of M^{3+} over M^{+} and M^{2+} in the water–ethanol mixture [38].

In our continuous effort to design sensor for various analytes [39–42], here in we synthesized a new imidazole functionalized anthracene based Schiff base compound **L** (Scheme 1), which is capable of showing selective remarkable colorimetric change as well as turn-on fluorescence response in the presence of trivalent metal cations Al^{3+}, Cr^{3+} and Fe^{3+} in 1:1 water–methanol mixture. The selective colorimetric and fluorescent responses were reported as a consequence of protonation of the organic probe in the medium. Unlike the previously reported trivalent cation sensors [36,38] we were able to demonstrate by fluorescence microscopic studies that our non-cytotoxic receptor **L** can detect M^{3+} by turn-on fluorescence response even in live model human cells (HeLa).

2. Experimental

2.1. General information and materials

All of the materials for synthesis were purchased from commercial suppliers and used without further purification. The absorption spectra were recorded on a Perkin-Elmer Lamda-25 UV–vis spectrophotometer using 10 mm path length quartz cuvettes in the range of 300–800 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 **L** was obtained using Waters Q-ToF Premier mass spectrometer. 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; qn = quintet, m = multiplet. For the cell culture experiments, Dulbecco's Modified Eagle Medium (DMEM), trypsin–EDTA and antibiotic solutions were procured from Sigma–Aldrich (USA). Fetal bovine serum (FBS) was procured from PAA Laboratories, USA.

2.2. Synthesis of *L*

In 30 ml methanol, 2 mmol of 9-anthracenealdehyde was added and stirred for 30 min to dissolve it completely. To this mixture, 2.1 mmol of 1-(3-aminopropyl) imidazole was added and the mixture was stirred for 12 h to complete the Schiff base reaction. Subsequently the solvent was evaporated to get a sticky product. 20 ml of diethyl ether was then added and stirred for 10 min; a yellow colored solid product appeared soon which was filtered and washed thoroughly by methanol. The product was dried by in a desiccator. The calculated yield of **L** was found to be 82%. ^{1}H NMR [400 MHz, $CDCl_3$, TMS, J (Hz), δ (ppm)]: 9.46 (1H, s), 8.52 (2H, t, J = 7.2), 8.05 (2H, d, J = 12), 7.57–7.48 (6H, m), 7.21 (1H, s, $CDCl_3$), 7.12 (1H, s), 7.02 (1H, s), 4.25 (2H, t, J = 6.8), 3.91 (2H, t, J = 6.4), 2.42 (2H, qn, J = 6.8). ^{13}C NMR [100 MHz, $CDCl_3$, TMS, δ (ppm)]: 161.82, 137.73, 131.75, 130.49, 130.22, 130.03, 129.48, 127.39, 125.79, 124.95, 119.39, 59.55, 45.282, 32.782. ESI-MS (positive mode, m/z) Calculated for $C_{21}H_{19}N_3$: 313.16 (100%), 314.16 (22.9%). Found: 314.1622 [($M+H^+$) 100%], 315.1924 [($M+H^+$) 34%].

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** (5×10^{-3} mol L^{-1}) was prepared in methanol. The solution of **L** was then diluted to 2.5×10^{-5} mol L^{-1} with CH_3OH/aqueous mixed solvent (1:1, v/v). In titration experiments, a quartz optical cell of 1 cm optical path length was filled with a 2 mL solution of **L** (2.5×10^{-5} mol L^{-1}) 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 mL of **L** solution (2.5×10^{-5} mol L^{-1}). For fluorescence measurements, excitation was provided at 365 nm, and emission was acquired from 390 nm to 700 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 509 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 of the ligand and various ligand–metal complexes was ascertained on cultured HeLa cells by a standard MTT assay as per the manufacturer instruction (Sigma–Aldrich, MO, USA). HeLa cells were initially grown in a 25 cm^2 tissue culture flask in DMEM medium supplemented with 10% (v/v) FBS, penicillin (100 μg/mL) and streptomycin (100 μg/mL) under a humidified atmosphere of 5% CO_2 in an incubator until the cells were approximately 90% confluent. Prior to MTT assay, cells were trypsinized and seeded into 96-well tissue culture plates at a cell-density of 10^4 cells per well and incubated with varying concentrations (12, 24, 36, 48, 60, 72, 84 and 96 μM) of the ligand, ligand in the presence of metal salts and metal salts made in methanol solvent (0.1%, v/v) and incubated for a period of 24 h under 5% CO_2. Solvent control samples (cells incubated in 0.1% methanol) were also included in parallel sets. Following incubation, the growth media was carefully removed, and fresh DMEM containing MTT solution was added to the wells. The plate was incubated for 4 h at 37 °C. Following incubation, 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. The assay was performed in six sets for each concentration of ligand, ligand in the presence of metal salts and metal salts. Data analysis and determination of standard deviation was performed with Microsoft Excel 2010 (Microsoft Corporation). In the MTT assay, the absorbance for the solvent control cells was