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A unique rhodamine-based 'off–on' molecular spy for selective detection of trivalent aluminum and chromium ions: Synthesis, crystal structure and spectroscopic properties along with living cell imaging

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ABSTRACT

The well known rhodamine framework offers an ideal model for the development of fluorescence enhanced chemosensors. Herein, a novel and simple molecule chemosensor, (E)-2-((benzo[d][1,3]dioxol-4-ylmethylene)amino)-3′,6′-bis(diethylamino)spiro[isoindoline-1,9′-xanthen]-3-one (**L**), has been designed by combining a rhodamine B hydrazide and a benzo[d][1,3]dioxole-4-carbaldehyde in a single molecule to prove the selectivity and sensitivity for Al^{3+} and Cr^{3+} in a dual-channel mode (fluorescence emission and UV–Vis). The signal transduction occurred by the increase of conjugation in the ring-open form than in the ring-closed form. Furthermore, the chemosensor **L** could also be used as an imaging probe without cytotoxicity for uptake and detection of Al^{3+} ion in HeLa cells.

1. Introduction

A minute quantity of metal ions are essential for living organisms, but excessive amount accumulated in the body causes permanent damage of several organisms. For example, Al^{3+} toxicity causes bone and joint diseases, neuronal disorder leading to dementia, myopathy, Alzheimer's disease and Parkinson's disease [1–5]. Acid rain increases free Al^{3+} in surface water by leaching from soil. This is deadly to growing plants [6]. According to a WHO report, the average human intake of aluminum is approximately 3–10 mg/day [7,8]. Chromium (III), on the other hand, plays an important role in effective metabolism of carbohydrate, lipid and protein by activating certain enzymes [9–12]. A deficiency of chromium in human body would lead to a variety of diseases, including diabetes and cardiovascular disease [13]. Exposure to high levels of Cr^{3+} can negatively inflict cellular structures [14–16]. Therefore, development of molecular sensors for the selective detection of traces of these metal ions are of considerable current research interest. Many sensors are so far reported for selective detection of Al^{3+} or Cr^{3+} [17–24]. Recently, based on the protocol of metal coordination inducing spiro-ring opening [25–30] of a sensing molecule, rhodamine B spirolactam was employed to design useful sensors for imaging environmentally and biologically active Al^{3+} and Cr^{3+} ions at physiological pH. But single sensors which can detect both Al^{3+} and Cr^{3+} ions selectively over other trivalent, divalent and monovalent ions are rare. There are some sensors suitable for sensing Al^{3+} with fluorescence turn-on process. Fluorescent turn-on reagents suitable for monitoring intracellular Cr^{3+} are countable and remain underdeveloped due to paramagnetic property and lack of a selective multi-chelating ligand for Cr^{3+} ion. It is more challenging to develop selective chemical sensors for these ions that can have naked eye sensitivity and/or which can give signal at the red region by "turn on" emission under physiological conditions after binding with target metal ion. Because red emitting sensors show minimal photo damage to biological samples and minimum interference from background autofluorescence in living systems [31–33]. We have developed sensor **L** that has several advantages over the others like a quick, simple and facile synthesis (without column purification). To the best of our knowledge, this is the first example of rhodamine based CHEF chemosensor at red region which can selectively sense both Al^{3+} and Cr^{3+} ions. Furthermore, biological application of **L** was evaluated for in vitro detection of Al^{3+} ion on HeLa cells of human cervical cancer with no significant cytotoxic effect.

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2. Experimental

2.1. Materials and instruments

All the solvents were of analytic grade. All reagents were purchased from Sigma–Aldrich and used without further purification. Elemental analyses (carbon, hydrogen and nitrogen) were performed with a Perkin–Elmer CHN analyzer 2400. Melting points were determined using a Buchi 530 melting apparatus. NMR spectra were recorded on a Bruker spectrometer at 500 (^{1}H NMR) MHz in DMSO-d_6. Chemical shifts (δ values) were reported in ppm down field from internal standard Me_4Si. Mass spectra were recorded in methanol solvent in Qtof Micro YA263. The electronic spectra were recorded in methanol–water solution on a Hitachi model U-3501 spectrophotometer. IR spectra (KBr pellet, 500–4000 cm^{-1}) were recorded on a Perkin–Elmer model 883 infrared spectrophotometer. Emission spectra were measured by Perkin Elmer (Model LS-50B) fluorimeter. Fluorescence lifetimes were measured by the method of Time Correlated Single-Photon Counting (TCSPC) using a HORIBA JobinYvon Fluorocube-01-NL fluorescence lifetime spectrometer. The sample was excited using a nanosecond laser diode at 340 nm and the signals were collected at the magic angle of 54.7° to eliminate any considerable contribution from fluorescence anisotropy decay [34]. The typical time resolution of our experimental set-up is ~800 ps. The decays were deconvoluted using DAS-6 decay analysis software. The acceptability of the fits was judged by χ^2 criteria and visual inspection of the residuals of the fitted function to the data. Mean (average) fluorescence lifetimes were calculated using the following Eq. (1):

$$\tau_{av} = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} \quad (1)$$

in which α_i is the pre-exponential factor corresponding to the ith decay time constant, τ_i.

2.2. Synthesis

Rhodamine B hydrazide was synthesized following the literature procedure [35,36] and was characterized by ^{1}H NMR spectra, mass data and FT-IR. To a 100 mL reactor, was charged 2-amino-3′, 6′-bis(diethylamino)spiro [isoindoline-1,9′-xanthen]-3-one (0.2 g, 0.438 mmol), benzo[d][1,3]dioxole-4-carbaldehyde (0.05 mL, 0.4 38 mmol) and methanol (10 mL). The reaction mixture was refluxed for overnight under oil bath at 90 °C. Then, the solution was cooled to room temperature, filtered and washed with ether and dried in vacuo over fused $CaCl_2$. A white crystal was obtained. Yield : 0.15 g, 58 %. M.P. −230 °C (decomp.). IR (KBr, cm^{-1}) 500–4000: ν(C=O) 1724.3, ν(C=N) 1616.2; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm), 1.09 (t, 12H, $NCH_2\underline{CH_3}$, J = 5 Hz), 3.32 (q, 8H, $N\underline{CH_2}CH_3$, J = 5 Hz), 6.03 (s, 2H, 1,3-dioxole), 6.35–6.33 (m, 2H, xanthene-H), 6.44–6.40 (m, 4H, xanthene-H), 6.79 (s, 1H, benzo-dioxole-H), 6.89–6.87 (m, 1H, benzo-dioxole-H), 6.96 (d, 1H, benzo-dioxole-H, J = 0.61 Hz), 7.10 (d, 1H, Ar-H, J = 7.5 Hz), 7.62–7.57 (m, 2H, Ar-H), 7.90 (d, 1H, Ar-H), 8.95 (s, 1H, N=C—H); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm), 12.87, 44.20, 66.10, 78.77, 79.20, 79.64, 97.93, 101.78, 106.07, 108.30, 109.54, 117.32, 121.93, 123.32, 124.18, 127.94, 128.98, 129.46, 134.00, 141.85, 146.84, 148.05, 148.90, 151.35, 153.30, 164.27; ESI-MS: m/z calculated for $C_{36}H_{37}N_4O_4$ $[M+H]^+$ 589.28, found 589.7. *Anal.* Calc. for $C_{36}H_{36}N_4O_4$: C, 73.57; H, 6.23; N, 9.56; Found: C, 73.45; H, 6.16; N, 9.52%.

2.3. Synthesis of **L**-Al^{3+} complex

A 2 mL methanolic solution of $Al_2(SO_4)_3{\cdot}16H_2O$ (0.053 g, 0.0850 mmol) was added dropwise to a magnetically stirred solution (5 mL) of **L** (0.05 g, 0.0850 mmol) in methanol. After two hours of stirring at room temperature, solvent was removed using a rotary evaporator, while a magenta colored precipitate was obtained. The complex was characterized by ^{1}H NMR, mass and FT-IR studies.

2.4. Crystallographic measurements

Measurements were done on a Bruker SMART APEX II CCD area detector equipped with graphite monochromated Mo Kα radiation (k = 0.71073 Å) source in ω scan mode at 296(2) K. The structures of the complexes were solved using the SHELXS-97 package of programs and refined by the full-matrix least square technique based on F^2 in SHELXL-97 [37]. All non-hydrogen atoms were refined anisotropically. Positions of the hydrogen atoms attached to carbon atoms were fixed at their ideal positions.

2.5. Fluorimetric analysis

For measurement of the quantum yields of **L** and its complex (in situ, after addition of 1 equivalent of each metal ion) with Al^{3+} and Cr^{3+} ions, we recorded the absorbance of the compounds in methanol/H_2O solution. The emission spectra were recorded using the maximal excitation wavelengths, and the integrated areas of the fluorescence-corrected spectra were measured. The quantum yields were then calculated by comparison with rhodamine B (Φ_s = 0.95 in ethanol) [38] as reference using the following equation [39]:

$$\Phi_X = \Phi_S \times (I_X/I_S) \times (A_S/A_X) \times (\eta X/\eta S)^2 \quad (2)$$

where, X and S indicate the unknown and standard solution, respectively, Φ is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance and η is the refractive index of the solvent.

2.6. Association constant

(i) Calculations for the binding constants using spectrophotometric titration data

The binding constants for the formation of the respective complex, [Al^{3+}-**L**] and [Cr^{3+}-**L**] were evaluated using the well known Benesi–Hildebrand (B−H) plot (Eq. (3)) [40].

$$1/(A - A_0) = 1/\{K(A_{max} - A_0)C\} + 1/(A_{max} - A_0) \quad (3)$$

A_0 is the absorbance of **L** at absorbance maxima (λ = 558 nm for Al^{3+} and Cr^{3+}), A is the observed absorbance at that particular wavelength in the presence of a certain concentration of the metal ion (C), A_{max} is the maximum absorbance value that was obtained at λ = 558 nm (for Al^{3+} or Cr^{3+}) during titration with varying [C], K is the association constant (M^{-1}) and was determined from the slope of the linear plot, and [C] is the concentration of the Al^{3+} or Cr^{3+} ion added during titration studies. The goodness of the linear fit of the B−H plot of 1/ $(A - A_0)$ versus 1/[Al^{3+}] or 1/[Cr^{3+}] for 1:1 complex formation confirms the binding stoichiometry between **L** and Al^{3+} or Cr^{3+}.

(ii) Calculations for the binding constants using emission titration data

$$1/(I - I_0) = 1/K(I_{max} - I_0)[C] + 1/(I_{max} - I_0) \quad (4)$$

Binding stoichiometries for the respective complex formations were also confirmed from Job's Plot. In the case of evaluation of the binding constant from the results of fluorescence titration, a modified B−H equation (Eq. (4)) was used, where I_0, I_{max}, and I represent the emission intensity of free **L**, the maximum emission