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First rhodamine-based "off–on" chemosensor with high selectivity and sensitivity for Zr^{4+} and its imaging in living cell

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ABSTRACT

A new pyridine–thiophene appended rhodamine-based probe RhPT was synthesized as "off–on" chemosensor for Zr^{4+}. Rhodamine spirolactam or spirolactone derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam/lactone gives rise to strong fluorescence emission and a pink color. However, in the present case, chemosensor RhPT shows high sensitivity and selectivity toward Zr^{4+} ions by exhibiting both colorimetric and fluorescence responses in CH_3OH–water (4:1, v/v, 10 μM HEPES buffer, pH 7.4). The selectivity of RhPT to the various metal ions was investigated. A color change and marked enhancement of fluorescence was found in the presence of Zr^{4+} due to the ring open reaction of rhodamine. The sensor shows extremely high fluorescence enhancement upon complexation with Zr^{4+} and it can be used as a "naked eye" sensor. The visual detection is possible by a sharp change in color. In addition, the turn-on fluorescent probe upon the addition of Zr^{4+} was applied in live cell imaging.

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1. Introduction

The recognition and sensing of various transition-metal ions are vital for the biological and environmental important species [1,2]. Among these is the zirconium ion, which is widely used for the preparation of laboratory crucibles, metallurgical furnaces, as a refractory material, sintered, ceramic knife, jewelry and nuclear appliances. However, their frequent use can result in high levels of residual zirconium ions, which may result in the contamination of water systems and soil and therefore cause a health hazard. Some toxic effects of zirconium ion have been reported, with symptoms limited mostly to gastrointestinal complaints such as nausea, abdominal pain and vomiting [3]. Inhalation of zirconium compounds can cause pulmonary granulomas, skin and lung granulomas. However, we have little other knowledge of the role of zirconium in human metabolism due to the lack of effective tools with which to study the mechanisms. Therefore, it is important to develop highly sensitive and selective methods that would be very useful for the real-time monitoring of the metal ions in environmental and biological samples. In recent years, significant emphasis has been placed on the development of new, highly selective fluorescent chemosensors of different architectures for metal cations because of their potential applications in biochemistry and environmental research [4].

Fluorogenic methods in conjunction with suitable probes are preferable approaches for the measurement of these analytes because fluorimetry is rapidly performed, nondestructive, highly sensitive and suitable for high-throughput screening applications [5]. In addition, colorimetric and/or fluorescent probes for the determination of transition metal cations have great popularity because they can monitor analytes both in solution by the naked eye [6] and in living cells by fluorescent microscopy [7]. To our knowledge, however, there is no report of a fluorescent probe used as a selective Zr^{4+} sensor. As the continuation of our work on the sensing of cations [8] and anions [9] of biological significance, herein, we report the systematic investigations of rhodamine based novel chemosensor RhPT with different metal binder scaffold and sensing behavior that combine a 6-thiophenyl-2-pyridinecarboxaldehyde and a rhodamine chromophore (Scheme 1). Rhodamine B is widely used as a fluorescent probe [10] for the detection of cysteine [11] and metal ions [12], including Cu^{2+} [13], Cr^{3+} [14], Fe^{3+} [15] and Hg^{2+} [16] due to the ring opening reaction of rhodamine. Rhodamine spirolactam or spirolactone derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam/lactone gives rise to strong fluorescence emission and a pink color. However, in the present case, chemosensor RhPT shows high sensitivity and selectivity toward Zr^{4+} ions by exhibiting both colorimetric and fluorescence responses in CH_3OH–water (4:1, v/v,

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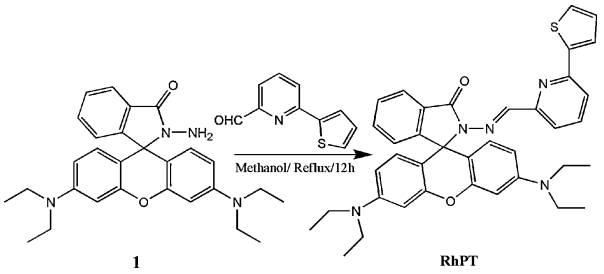

Scheme 1. Synthetic route of **RhPT**.

10 μM HEPES buffer, pH 7.4). This rhodamine B derivative (RhPT) can selectively detect Zr^{4+} in the presence of other metal ions when ethylenediaminetetraacetic acid (EDTA) is used as a second ligand, and thus be used as a Zr^{4+} sensor in living cells. As far as we are aware, RhPT is the first chemosensor for zirconium based on rhodamine derivative.

2. Experimental

2.1. Materials and measurements

All the solvents were of analytic grade. All cationic compound such as per chlorate of Hg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, nitrate of Y^{3+}, Ag^{+}, chlorides of Ti^{4+}, Pd^{2+} and $ZrOCl_2{\cdot}8H_2O$ were purchased from Sigma–Aldrich Chemical Co., stored in a desiccators under vacuum containing self-indicating silica, and used without any further purification. Solvents were dried according to standard procedures. Unless stated otherwise, commercial grade chemicals were used without further purification. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Spectrochem GF254 silica gel coated plates. The ^{1}H NMR spectra were recorded on Bruker-AM-400 spectrometer. The ^{1}H NMR chemical shift values are expressed in ppm (δ) relative to $CHCl_3$ (δ = 7.26 ppm). UV–visible and fluorescence spectra measurements were performed on a JASCO V530 and a Photon Technology International (LPS-220B). A 1.0×10^{-5} M solution of the probe RhPT in MeOH/H_2O (4:1, v/v) was prepared and stored in the dry atmosphere. Solutions of 2.0×10^{-4} M salts of the respective cation were prepared in analytic grade MeOH and were stored under a dry atmosphere. All experiments were carried out in CH_3OH–H_2O solution (CH_3OH:H_2O = 4:1, v/v, 10 μM HEPES buffer, pH = 7.4). Binding constant was calculated according to the Benesi–Hildebrand equation. K_a was calculated following the equation stated below: $1/(A - A_o) = 1/K(A_{max} - A_o)[M^{n+}] + 1/(A_{max} - A_o)$. Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest (M^{n+}), A_{max} is the maximum absorbance value that was obtained at λ = 563 nm during titration with varying (M^{n+})and K is the association constant (M^{-1}). The association constant (K) could be determined from the slope of the straight line of the plot of $1/(A - A_o)$ against $1/[M^{n+}]$. The binding constant value of Zr^{4+} with receptor has been determined from the emission intensity data following the modified Benesi–Hildebrand equation. $1/I - I_0 = 1/K(I_{max} - I_0)[M^{n+}] + 1/(I_{max} - I_0)$, where I_0, I_{max} and I represent the emission intensity of free receptor, the maximum emission intensity observed in the presence of added metal ion Zr^{4+} at 582 nm (λ_{ext} = 563 nm) and the emission intensity at a certain concentration of the metal ion added, respectively.

2.2. Cell culture and fluorescence imaging

Candida albicans cells (IMTECH No. 3018) and Pollen cells from exponentially growing culture in yeast extract glucose broth medium (pH 6.0, incubation temperature, 37 °C) were centrifuged at 3000 rpm for 10 min, washed twice with 0.1 M HEPES buffer at pH 7.4. Then, cells were first incubated with 20 μM of sensor RhPT (in the MEM (modified Eagle's medium) culture medium containing 49:1, v/v, water–ethanol) for 30 min at 37 °C in two different cover glass bottom dish containing 0.01% Triton X100 as permeability enhancing agent and then washed with PBS (containing 2.0% methanol) (0.1 M, pH = 7.4) three times to remove excess of RhPT. After incubation the cells, it was observed under a Leica DM 1000 fluorescence microscope equipped with UV filter. Then Zr^{4+} (0, 20, 50 mM) (aqueous solution) was added to the specimen at the point of observation with the help of a micropipette at 37 °C. Cell imaging was then carried out after washing cells with physiological saline. For the method of cell toxicity determination please see SI, pp. S12–S14.

2.3. Synthesis of chemosensor RhPT

A solution of rhodamine B hydrazide (1, 0.40 g, 0.88 mmol) and 6-thiophenyl-2-pyridinecarboxaldehyde (0.17 g, 0.89 mmol) in 20 ml of dry methanol was refluxed for 12 h. After that, the solution was cooled (concentrated to 10 ml) and allowed to stand at room temperature overnight. The precipitate which appeared next day was filtered and washed 4 times with 10 ml cold ethanol. After drying under reduced pressure, the reaction afforded, 0.47 g RhPT as white solid. Yield: 85%; m.p. 214–216 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.79 (s, 1H, —CH=N), 8.06 (d, 1H, J = 6.9 Hz), 7.58–7.53 (m, 5H), 7.34 (d, 2H, J = 3.84 Hz), 7.07 (t, 2H, J = 5.4 Hz), 6.50 (dd, 4H), 6.26 (s, 2H), 3.33 (q, 8H, —NCH_2CH_3), 1.16 (t, 12H, —NCH_2CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm): 12.71, 29.83, 44.56, 66.09, 98.21, 104.63, 108.66, 118.21, 119.04, 124.11, 125.85, 125.79, 127.81, 128.07, 128.38, 128.66, 129.42, 134.02, 137.01, 137.84, 144.56, 152.17, 153.15, 154.21, 164.63. TOF MS ES$^+$, m/z = 628.10 [M]$^+$, calc. for $C_{38}H_{37}N_5O_2S$ = 627.77. Anal. calcd. for $C_{38}H_{37}N_5O_2S$: C, 72.70; H, 5.94; N, 11.16; O, 5.09; S, 5.11 Found: C, 72.42; H, 5.67; N, 10.89; O, 4.81; S, 5.38.

3. Results and discussion

3.1. UV–vis and fluorescence spectra of chemosensor RhPT

As shown in Scheme 1, the rhodamine B derivative RhPT was prepared in 85% yield as white solid by reacting 1 [17] with 6-thiophenyl-2-pyridinecarboxaldehyde in an equal molar ratio in methanol. The structure of RhPT was verified by ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses (see Supporting information, pp. S7–S9).

Chemosensor RhPT was designed to bind metal ions via the carbonyl O, enamine N, pyridyl N and thiophene S as donors. All the spectroscopic studies were performed in 4:1 CH_3OH–water in which chemosensor RhPT formed a colorless solution that was stable for more than four month. The solution of compound RhPT was very weakly fluorescent in the absence of any analyte due to the predominant ring-closed spirolactone and compound showing no absorption at visible region. Absorption and fluorescence titrations of RhPT were conducted in methanol–water (4:1, v/v) solution. The spectroscopic properties of the chemosensor RhPT toward the metal ions such as Zr^{4+}, Y^{3+}, Ti^{4+}, Hg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, $Pd^{2+}$and Ag^{+} were used in CH_3OH–H_2O solution (CH_3OH:H_2O = 4:1, v/v, 10 μM HEPES buffer, pH = 7.4). In this context, a higher content of water is seemed to be desirable for better practical applicability of the sensor. But we cannot use much more water due to limited solubility of RhPT in water.

Emission and absorption titrations of RhPT were monitored in CH_3OH–H_2O (4:1, v/v, 10 μM HEPES buffer, pH = 7.4). Probe RhPT displays very weak spectral characteristics in emission spectrum

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