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Probing chromium(III) from chromium(VI) in cells by a fluorescent sensor

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ABSTRACT

Cellular uptake of Cr(VI), followed by its reduction to Cr(III) with the formation of kinetically inert Cr(III) complexes, is a complex process. To better understand its physiological and pathological functions, efficient methods for the monitoring of Cr(VI) are desired. In this paper a selective fluorescent probe **L**, rhodamine hydrazide bearing a benzo[b]furan-2-carboxaldehyde group, was demonstrated as a red chemosensor for Cr(III) at about 586 nm. This probe has been used to probe Cr(III) which is reduced from Cr(VI) by reductants such as glutathione (GSH), vitamin C, cysteine (Cys), H_2O_2 and Dithiothreitol (DTT) by fluorescence spectra. Cr(VI) metabolism in vivo is primarily driven by Vc and GSH. Vc could reduce CrO_4^{2-} to Cr(III) in a faster rate than GSH. The indirectly detection limit for Cr(VI) by **L** + GSH system was determined to be 0.06 μM at pH = 6.2. Moreover, the confocal microscopy image experiments indicated that Cr(VI) can be reduced to Cr(III) inside cells rapidly and the resulted Cr(III) can be captured and imaged timely by **L**.

1. Introduction

Chromium(VI) is a potent, well-established carcinogen [1], present in the environment as an air and soil pollutant [2,3]. Cr(VI) is easily taken inside the cells through membrane channels meant for the transfer of the isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-} [4]. Cellular uptake of Cr(VI), followed by its reduction to Cr(III) with the formation of reactive intermediates Cr(V/IV), is a generally accepted cause of Cr(VI)-induced genotoxicity and carcinogenicity. Cellular reductants such as ascorbic acid (Vc) [5] and small cellular thiol glutathione (GSH) and cysteine (Cys) [6] are able to reduce Cr(VI) to Cr(V/IV) and ultimately to Cr(III) form [7]. Vc is a dramatically faster reducer of Cr(VI) than thiols in vitro, and it is responsible for the overwhelming majority of chromate metabolism in the lung, kidney and liver [8–10]. In recent years, it has also been proposed that hydrogen peroxide might collaborate with other species presented in the cellular medium (mainly with thiols such as glutathione [11,12]) in the activation of chromium(VI) required to produce its toxic and carcinogenic effects [13–17]. By contrast, most nutritionists regard Cr(III) as an essential micronutrient [18,19], acting as an insulin activator, although this opinion has been disputed [20,21].

To understand the chemical basis of Cr biological activities, many methods have been applied, such as global kinetic analysis techniques [22], X-ray absorption spectroscopy, electrospray mass spectrometry and EPR spectroscopy [23]. Even this, only few direct spectroscopic evidences have been obtained for the reduction process of Cr(VI) in cells [24,25]. A convenient and rapid method for the analysis of Cr(VI)/Cr(III) in cells is urgently demanded. In the present work, a simple rhodamine hydrazide derivative **L** bearing a benzo[b]furan-2-carboxaldehyde group has been demonstrated as a probe for Cr(III). This probe can be used to detect the reduction of chromate in cells using fluorescence spectroscopy.

2. Experimental

2.1. Materials and instruments

All the reagents were analytical grade and used as received. All solvents were used after appropriate distillation or purification. Deionized water was used throughout the experiments. K_2HPO_4 buffer solutions (0.01 M, pH 6.2) were prepared in water. The solutions of metal ions were prepared from nitrates salts of K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Mn^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+} and Ba^{2+}, respectively. Dithiothreitol (DTT), vitamin C (Vc), H_2O_2, cysteine (Cys), NO_2^- and ClO_4^- solutions were always freshly prepared in K_2HPO_4 buffer solution (0.01 M K_2HPO_4, pH 6.2) and kept on ice. Colon cancer cell SW480 was obtained from the American Type Culture Collection. The medium and heat-inactivated fetal bovine serum (FBS) was from GIBCO (Grand Island, NY).

^1H NMR and ^{13}C NMR spectra were recorded on Bruker-400 MHz spectrometers, the chemical shifts (δ) were reported as ppm in DMSO-d_6. Elemental analyses were measured on a Vario EL III analyzer. ESI-MS spectra were conducted by Agilent 6520 Accurate-Mass Q-TOF LC/MS mass spectrometer. Fluorescence responses were recorded on Fluoromax-X spectrofluorometer (HORIBA). UV–visible (UV–vis) spectra were measured with a Varian 50 BIO spectrophotometer. All pH measurements were measured with a pH meter (Mettler Toledo,

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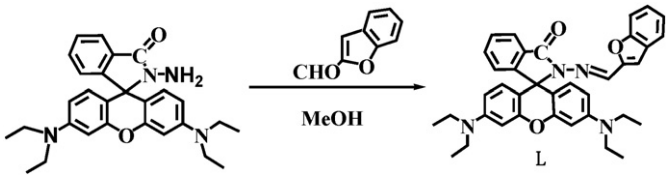


Scheme 1. Scheme 1 Synthetic procedures for probe L.

Switzerland). Bioimaging of the probes were performed by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope.

2.2. Synthesis of **L**

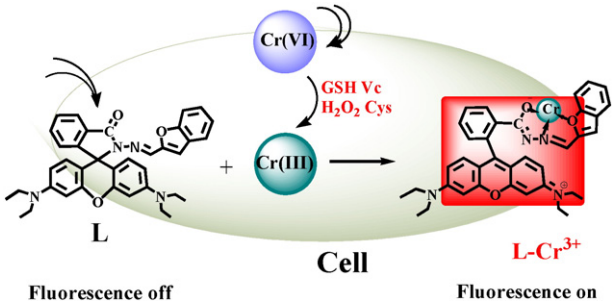
The synthetic route used to obtain compound **L** is shown in Scheme 1. Compound **L** was synthesized in one step by reaction of rhodamine hydrazide with benzo[b]furan-2-carboxaldehyde. Rhodamine hydrazide (0.46 g, 1 mmol) and benzo[b]furan-2-carboxaldehyde (0.584 g, 4 mmol) were dissolved in 20 mL absolute ethanol and then the solution was refluxed for 6 h. After cooling the precipitate was filtered and washed with methanol for three times. The crude product **L** was further purified by chromatography on silica gel (Petroleum ether/ethyl acetate = 5:2, v/v) to give the white solid. ESI mass spectrometry: m/z = 585.28 $[M + H]^+$, $[M + H]^+$ calculated 585.28. ^{13}C NMR (DMSO-d_6) δ (ppm): 164.4, 154.6, 152.4, 151.9, 150.9, 148.5, 137.9, 134.1, 128.8, 127.6, 123.6, 123.1, 121.9, 111.5, 110.9, 108.1, 105.0, and 97.5. ^1H NMR(DMSO-d_6), δ (ppm, 300 MHz, TMS): δ 8. 94 (s, 1H, N = C–H), 7.93 (d, 1H), 7.60 (m, 4H, Phen–H), 7.35 (t, 1H, Phen–H), 7.26 (t, 2H, Phen–H), 7.06 (d, 1H), 6.46 (d, 4H, Xanthene–H), 6.37 (2H, Xanthene–H), 3.33 (q, 8H, NCH_2CH_3), and 1.08 (t, 12H, NCH_2CH_3) (Figs. S1–S3).

2.3. UV–vis and fluorescence titration study

Reduction of Cr(VI) to Cr(III) was monitored by UV–vis absorbance and fluorescence spectra of the probe **L**. The nitrates salts of K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Mn^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}, Ba^{2+} and K_2CrO_4 was dissolved in K_2HPO_4 buffer solutions (0.01 M, pH 6.2) to afford 1.0 mM solution. The 1.0 mM stock solution of **L** was prepared in absolute methanol. The spectral properties of **L** (10 μM) were investigated in 0.01 M EtOH–K_2HPO_4 solution (1:1, v/v, pH 6.2). Fluorescence measurements were carried out with excitation and emission slit width of 2.5 and 2.5 nm and PMT Voltage (photomultipler tube) and excitation wavelength was 540 nm.

2.4. Cell culture and fluorescence microscopy imaging

SW480 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS, and 1% penicillin–streptomycin at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air. Before imaging experiment, the cells were seeded in 24-well plates for 24 h.



Scheme 2. Proposed sensoring mechanisms for Cr(VI) by L in cell.

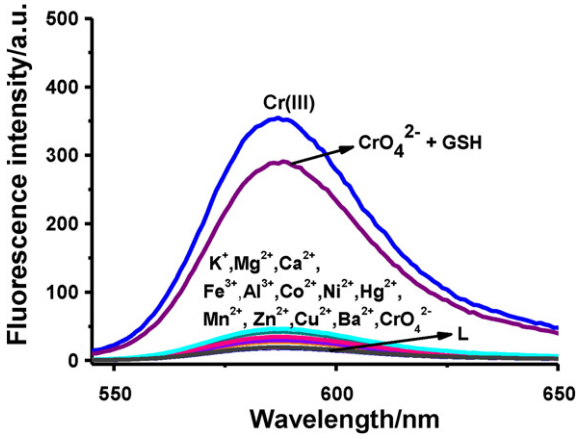


Fig. 1. Fluorescence spectra of **L** (20 μM) in the presence of 100 μM various metal ions (K^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Mn^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}, Ba^{2+}, CrO_4^{2-} or CrO_4^{2-} + 1.0 mM GSH) in EtOH–K_2HPO_4 solution (0.01 M K_2HPO_4, 1:1, v/v, pH 6.2), respectively. λ_{ex}: 540 nm.

The cells were incubated with **L** (20 μM) at 37 °C under 5% CO_2 for 30 min, washed 3 times to remove the remaining probe and bathed in DMEM containing no FBS prior to imaging. Then 20 μM CrO_4^{2-} was added in the growth medium for 0.5 h at 37 °C, washed 3 times with PBS (phosphate buffered saline) buffer. Then, cells were imaged under a laser confocal fluorescence imaging.

3. Results and discussion

3.1. Fluorescent recognition of Cr^{3+}

Rhodamine B is widely used as fluorescent probes in routine optical analysis. Spirolactam-type rhodamine derivatives are nonfluorescent and colorless, whereas spirolactam ring-opening gives rise to strong fluorescence emission and pink color [26]. To explore the sensitivity of the probe **L**, fluorescence responses of **L** to various metal ions were carried out. As illustrated by Fig. 1, **L** (20 μM) exhibited very weak fluorescence in EtOH–K_2HPO_4 solution (1:1, v/v, pH 6.2, λ_{ex} = 540 nm). Addition of 100 μM Cr^{3+} led **L** to fluorescence with a 10-fold enhancement at about 586 nm. Meanwhile, addition of 100 μM Fe^{3+} and Al^{3+} caused a very weak increase for probe **L**. No obvious responses could be observed upon the addition of 100 μM K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Ba^{2+} and CrO_4^{2-} to **L**. The common coexistent ions had negligible interfering effect on Cr^{3+} sensing by **L** in EtOH–K_2HPO_4 solution (Fig. S4). It indicated that **L** had a higher sensitivity and excellent selectivity for Cr^{3+} in EtOH–K_2HPO_4 solution (1:1,

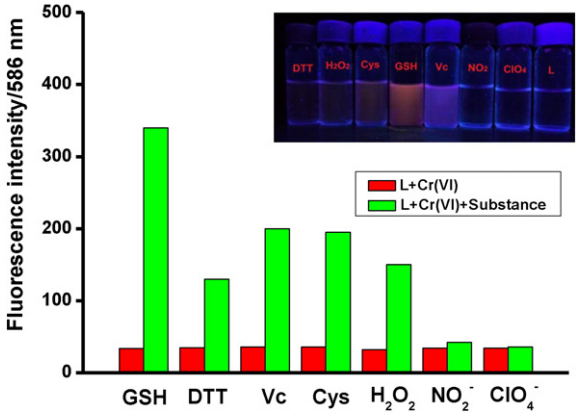


Fig. 2. Fluorescence intensity of **L** (20 μM) + Cr(VI) (100 μM) system in the absence/presence of 1 mM GSH, DTT, H_2O_2, Vc, Cys, NO_2^- and ClO_4^- in EtOH/K_2HPO_4 solution (0.01 M K_2HPO_4, 1:1, v/v, pH 6.2), λ_{ex} = 540 nm.