Sensors and Actuators B 174 (2012) 312–317

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

A new rhodamine–chitosan fluorescent material for the selective detection of Hg^{2+} in living cells and efficient adsorption of Hg^{2+} in natural water

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ARTICLE INFO

Article history:
Received 10 April 2011
Received in revised form 22 February 2012
Accepted 23 March 2012
Available online 15 June 2012

Keywords:
Probe
Colorimetric
Fluorescence imaging
Chitosan
Absorbent

ABSTRACT

A new rhodamine-based fluorescence probe (AG-RB) is reported. This probe exhibits favorable cell-permeable due to the introduction of D-glucosamine and has been successfully used to detect intracellular Hg^{2+} by fluorescence imaging method. The modified-rhodamine chitosan material (CS-RB) is able to recognize and adsorb Hg^{2+} by spectroscopic and "naked-eye" method in water.

1. Introduction

Hg^{2+} is one of the most serious environmental and health threats in the vast decays due to their increasing utilization in industry and agriculture [1–7]. Exposure of mercury even at low concentration leads to digestive, kidney and neurological diseases [8–10], especially. These environmental and health problems have prompted the development of methods for the detection and quantification of mercury applicability, especially in situations where conventional techniques are not appropriate. While significant progress in the creation of highly sensitive chromogenic, fluorogenic and redox probes has been achieved [11–17], the mercury-indicating methodologies, which are developed to provide critical information for mercury hazard assessment and mercury pollution management, are in still in high demand. Many materials have been used as adsorbent for heavy metal ion in wastewater, however, their used analysis methods, such as atomic absorption spectrophotometer, FT-IR spectra, etc., are complicated, expensive and indirect-view [18,19].

In our previous work, we developed an efficient strategy for preparing fluorescent turn-on chemosensor **RG1** by incorporating the glucose and rhodamine 6G into one molecule [20], **RG1** maintained the excellent water solubility and favorable biocompatibility as well as the high-sensitivity toward Hg^{2+} with the fluorescence enhancement manner, benefiting the practical application for screening Hg^{2+} in natural water and living cells. Since D-glucosamine is one of the most abundant monosaccharide [21], and an important material for the synthesis of antibiotics and anticancer drugs [22–24]. The outstanding biocompatibility and water-solubility of glucosamine make it a desirable to design of cell-permeable small molecular fluorescence probes that conveniently used as biological reporters in living cells and even in whole living animals [25–29]. Its polymer pattern, chitosan, that derived from deacetylation of chitin (the abundance is second only to cellulose among polysaccharine found on Earth) [30] have also been widely studied in environment [31–33], drug delivery [34,35], optical devices [36–39] and biomedical fields [40].

By coupling the D-glucosamine block and rhodamine B unit within one molecule, we report here a fluorescent probe that exhibits high sensitivity for selective detection of Hg^{2+} in water and excellent cell-permeable for the imaging of Hg^{2+} in living cells. Chitosan-based material, **CS-RB**, was also prepared through a normal immobilization approach with the aim to further realize the environmental applications of these probes in absorption of Hg^{2+}. We envisioned that **CS-RB** could work as excellent Hg^{2+} probe with a highly selective and sensitive by fluorimetric and colorimetric method, at the same time, it was able to remove Hg^{2+} from water in natural environment.

2. Experimental

2.1. Reagents and chemicals

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. D-Glucosamine hydrochloride and chitosan were purchased from Sinopharm

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0925-4005/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.snb.2012.03.072

Chemical Reagent Co., Ltd. (China); Rhodamine B was obtained from Aldrich. Stock solution (2×10^{-2} M) of the aqueous nitrate salts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+} were prepared for further experiments.

2.2. Instruments and spectroscopic measurements

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova-400 spectrometer with chemical shifts reported as *ppm* (in CD_3Cl, TMS as internal standard). Mass spectral determinations were made on an ESI-Q-TOF mass spectrometry (Micromass, UK). High resolution mass spectra measurements were performed on a GC–TOF mass spectrometry (Micromass, UK). FT-IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer equipped with a Nic-Plan Microscope. UV–vis diffuse reflectance spectra were taken on a Shimadzu UV-2401PC spectrophotometer using $BaSO_4$ as the reference. Elemental analyses (C, H, and N) were performed on an Elementary Vario EL analyzer. Fluorescence spectra were determined with FS920 luminescence spectrometer (Edinburgh Instruments). Absorption spectra were measured with Lambda 35 UV-*vis* spectrophotometer. All pH measurements were made with a Model PHS-3C meter. The adsorption ability of **CS-RB** for Hg^{2+} in water was measured by Inductively Coupled Plasma Spectrometer (Perkin Elmer). Cells were imaged by Nikon eclipse TE2000-5 inverted fluorescence microscopy.

2.3. Synthesis of *AG-RB*

RB-DQ [41] (0.99 g, 2 mmol) was dissolved in 30 ml methanol. To this solution D-glucosamine·HCl (0.43 g, 2 mmol) and triethylamine (0.3 ml) were added. The mixture was refluxed for 90 min under N_2. After cooling to room temperature, the solvent was removed under reduced pressure. The crude product was then purified by chromatography on a silica gel column (CH_2Cl_2:CH_3OH, 1:15, V/V) to give **AG-RB** as a yellow solid in 45% yield. Elemental Analysis: calcd (%) for $C_{36}H_{43}N_5O_7$: C 65.74, H 6.59, N 10.65. Found (%): C 65.56 H 6.62 N 10.61. ESI-MS negative peak at *m/z* 692.2 indicated [**AG-RB** + Cl]$^-$. ^{1}H NMR (DMSO-d6): 8.22 (d, 1H), 7.92 (d, 1H), 7.58 (d, 2H), 7.04 (d, 1H), 6.44 (m, 4H), 6.34 (d, 2H), 4.90 (d, 1H), 4.73 (d, 1H), 4.59 (t, 1H), 4.52 (t, 1H), 3.664 (m, 1H), 3.610 (m, 2H), 3.45 (m, 1H), 3.14 (m, 1H), 3.07 (t, 1H), 3.34 (m, 8H), 1.09 (t, 12H). ^{13}C NMR (DMSO-d6): 12.89, 44.11, 61.65, 65.65, 70.91, 74.82, 77.33, 78.66, 92.66, 95.63, 105.03, 108.78, 123.78, 124.19, 127.62, 127.98, 129.28, 134.96, 149.15, 152.57, 152.73, 162.57, 164.96.IR (KBr): 2973, 2893, 1705, 1635, 1616, 1516, 1377, 1305, 1266, 1233, 1118, 1049, 880.

2.4. Synthesis of *CS-RB*

RB-DQ (0.99 g, 2 mmol) was dissolved in 50 ml methanol. Then chitosan (2.0 g) was added. The mixture was refluxed for 8 h under N_2. After cooling to the room temperature and evaporated the solvent in vacuum. The resulting powder was then put into a Soxhlet's extractor and extracted with methanol for at least 12 h to ensure that there was noncovalently bounded **RB-DQ** in chitosan. After drying under reduced pressure, the reaction afforded **CS-RB** as yellow solid. Elemental Analysis: Found (%): C 42.69, H5.58, and N 6.65. IR (KBr): 2970, 2872, 1701, 1636, 1616, 1547, 1514, 1467, 1425, 1384, 1304, 1263, 1218, 1152; 1117, 1074.

2.5. Imaging of HeLa cells incubated with *AG-RB* and Hg^{2+}

HeLa cells were incubated with **AG-RB** (10 μM, in PBS medium) for 15 min at 37 °C. The remaining **AG-RB** was washed away with PBS. HeLa cells were then incubated with Hg^{2+} (0.2 mM) for another 15 min, and were imaged by fluorescence microscopy.

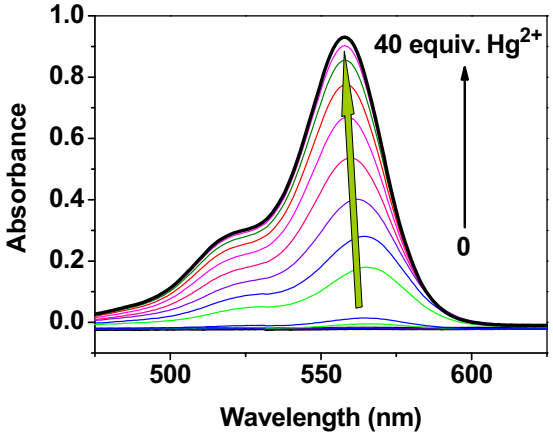

Fig. 1. UV–vis spectra of **AG-RB** (10 μM) in CH_3CN aqueous solution (30:70, v/v) in the presence of different amounts of Hg^{2+} (0–0.4 mM).

2.6. Adsorption ability of *CS-RB* for Hg^{2+}

CS-RB (50 mg) and chitosan (50 mg) were added to the about 1 *ppm* Hg^{2+} solution (50 ml), respectively. The mixture was stirred for 4 h. After filtration, the concentration of residual Hg^{2+} in the filtrate was analyzed by inductively coupled plasma source mass spectrometer (ICP).

3. Results and discussion

3.1. Synthesis and characterization of *AG-RB* and *CS-RB*

As described in Scheme 1, **AG-RB** and **CS-RB** were easily synthesized from the simple reaction of rhodamine hydrazide and glyoxal, followed by the reaction with D-glucosamine and chitosan, respectively. The structure of **AG-RB** was confirmed by ^{1}H NMR, ^{13}C NMR and ESI-MS. The characterization of **CS-RB** by elemental analysis (EA), UV–vis diffuse-reflectance spectra, and FT-IR spectra indicating that the rhodamine dye was successfully grafted onto chitosan material by C=N bond. We next investigate the optical properties of **AG-RB** upon Hg^{2+} and the absorption capability of **CS-RB** for Hg^{2+} in water.

3.2. The detection ability of *AG-RB* to Hg^{2+} in water and living cells

The absorption spectra of **AG-RB** (10 μM) in CH_3CN aqueous solution (30:70, v/v, pH = 7.0) exhibited a very weak absorption peak at about 564 nm, which was ascribed to the slight amounts of ring-open delocalized xanthenes forms of **AG-RB**. Upon the addition of 40 equiv. of Hg^{2+}, the absorbance was significantly enhanced with a continuous blue shift of about 7 nm (Fig. 1). These results demonstrated that the formation of the ring-opened form of rhodamine B moiety in **AR-RB** upon Hg^{2+} binding [42]. Meantime, the titration solution exhibited an obvious and characteristic color change from yellow to pink, indicating that **AG-RB** can serve as a "naked-eye" Hg^{2+} indicator in aqueous media. No significant adsorption and color changes occurred in the presence of alkali or alkaline, earth metals and the first-row transition metals, such as Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Ag^+ and Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^+, K^+, indicating the high selectivity of **AG-RB** toward Hg^{2+} (Fig. 2).

The solution of **AG-RB** (10 μM) showed very weak fluorescence. However, upon addition of Hg^{2+} (0.4 mM), the solution showed strong red fluorescence with an approximately 180-fold enhancement in the fluorescence intensity attributable to the