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Dye-functional mesoporous silica material for fluorimetric detection of Cr(III) in aqueous solution and biological imaging in living systems

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ABSTRACT

A dye-functionalized silica nanomaterial, SBA-RT was prepared by the immobilization of the Rhodamine-based chemosensor R6G-TETA within the channels of SBA-15. SBA-RT exhibits several different properties compared to the free R6G-TETA, such as higher selectivity, blue-shift of the UV–vis spectra due to special spatial environment in the channels of the mesoporous material. It presents Cr(III)-selective fluorimetric and colorimetric responses in aqueous solution. The fluorescence responses are reversible by treating with EDTA and do not vary over a broad pH range suitable for Cr(III) bioimaging application. Through isolating of the metal ions within the mesopores of the silica, SBA-RT can extract Cr(III) from the solution with only trace amounts remaining. The fluorescence images experiment demonstrated the possibility of further application in monitoring Cr(III) in living cells and organisms.

1. Introduction

Recently, inorganic host materials have been widely used as support to design functional materials used in catalysis [1–4], and drug delivery fields [5–9]. Specially, mesoporous silica materials have attracted much attention for optical applications due to their excellent properties, such as high surface area, uniform porosity, optical transparency in the UV/vis range, favorable biocompatibility and mechanical robustness [10–17]. The solid chemosensors could be achieved by covalent grafting of fluorescent receptors onto the inner surface of mesoporous silica materials [18–20]. Recently, functionalized mesoporous materials have been also used as adsorbent for wastewater containing heavy metal ions by atomic absorption spectrometry and infrared spectrophotometry [21–23]. However, these analysis methods have the following disadvantages: complicated operation, expensive instruments, unsuitable for real-time detection, etc. It is still a challenge to develop novel multifunctional materials that can detect and adsorb heavy-transition metal (HTM) ions with visualized output signals (fluorescence or color responses, etc.).

Trivalent chromium, has great impact on the metabolism of carbohydrates, fats, proteins and nucleic acids based on modulation of the action of insulin through glucose tolerance factors (GTF) [24,25]. The appropriate chromium intake is 50–200 μg per day and its deficiency causes disturbances in glucose levels and lipid metabolism. However, exposure to high levels of Cr(III) can negatively affect cellular structures [26–29]. On the other hand, chromium is an environmental pollutant and its build-up due to various industrial and agricultural activities is a matter of concern [30]. Thus, there is an urgent need to study suitable methods capable of sensing of Cr(III) in biological and environmental system [31]. However, the development of Cr(III)-specific chemosensors for quantificational detection of Cr(III) by fluorimetric methods is still a big challenge due to the lack of selective multi-chelating ligands [32,33]. Cr(III) is also one of the most effective fluorescent quenchers known due to its paramagnetic nature which also makes it difficult to develop "turn-on" types of chemosensors [34,26]. Rhodamine framework is an ideal mode to construct OFF–ON fluorescent sensors due to its particular structural property (the equilibrium between the non-fluorescent spiro-cyclic form and the highly fluorescent ring-open form) [35,36]. Of these Rhodamine-based Cr(III) fluorescent sensors have been developed [37–40], R6G-TETA is one of the most sensitive probes that exhibits excellent selectivity towards Cr(III) in HEPES aqueous solution over other transitions metal ions [41]. Upon the addition of 5 equiv. of Cr(III), about 60-fold fluorescence enhancement of R6G-TETA at 552 nm was observed. However, whether the sensor can be applied in bioimaging was not further investigated.

Bearing this in mind, we chose the Cr(III)-specific fluorescent chemosensor R6G-TETA as molecular probe and immobilized it onto the inner surface of SBA-15. We envisioned that the

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combination of the fluorescence sensor and mesoporous material would generate a novel multifunctional material, which could take on specific fluorescence response and high adsorptivity for Cr(III) in water. The favorable biocompatibility of the mesoporous silica material is beneficial to utilize the hybrid material in living cells and *in vivo* to study the toxicity or bioactivity of Cr(III) in living organisms.

2. Experimental

2.1. Reagents and chemicals

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. SBA-15 was purchased from Jilin University High Technology Co. Ltd., 3-(triethoxysilyl)propylisocyanate was obtained from Aldrich. Triethylenetetramine and metal salts were provided by Shanghai Chemical Reagent Co., Ltd. (China). Stock solution (2×10^{-2} M) of the aqueous nitrate salts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Ba^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Al^{3+}, Fe^{3+} and Cr^{3+} were prepared for further experiments.

2.2. Instruments and spectroscopic measurements

^{1}H NMR was measured on a Varian INOVA 400 M spectrometer with chemical shifts reported as ppm ($CDCl_3$, TMS as internal standard). X-ray powder diffraction (XRD) patterns of the SBA-15, SBA-RT were recorded on a Rigaku D/max-2400 X-ray powder diffractometer (Japan) using Cu Kα (λ = 1.5405 Å) radiation. Transmission Electron Microscope (TEM) images were taken on a Hitachi H-9000 NAR transmission electron microscope under a working voltage of 300 kV. 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. The nitrogen adsorption and desorption isotherms were measured at 77 K using an ASAP 2010 analyzer (Micromeritics Co. Ltd.). Surface areas were calculated by the Brunauer–Emmett–Teller (BET) method, and the pore volume and pore size distributions were calculated using the Barret–Joyner–Halenda (BJH) model. Fluorescence spectra of the solution were obtained using the FS920 spectrometer (Edinburgh Instruments). Both excitation and emission slit widths were 6 nm. Fluorescence measurements were carried out in a 2 cm quartzcuvette with stirring the suspension of SBA-RT. The adsorption ability of SBA-RT for Cr(III) in water was measured by inductively coupled plasma spectrometer (Perkin Elmer). HeLa Cells and zebrafish were imaged by Nikon eclipse TE2000-5 inverted fluorescence microscopy.

2.3. Synthesis of R6G-TETA

Compound R6G-TETA was prepared according to reported procedures [41]. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.92 (d, 1 H), 7.45 (t, 2 H), 7.043 (t, 1 H), 6.34 (s, 2 H), 6.21 (s, 2 H), 3.52 (t, 2 H), 3.22 (t, 4 H), 2.76 (m, 2 H), 2.61 (t, 2 H), 2.51 (m, 2 H), 2.35 (m, 4 H), 1.89 (s, 6 H), 1.32 (t, 6 H).

2.4. Synthesis of SBA-IPTES

3-(Triethoxysilyl)propylisocyanate (IPTES) (0.247 g, 1 mmol) and SBA-15 (1.0 g) were suspended in anhydrous toluene (50 mL) and stirred in reflux condition under N_2 for 24 h. The precipitate formed was filtered, and adequately washed several times with toluene and CH_2Cl_2 to rinse away any surplus IPTES. A white powder was obtained and denoted as SBA-IPTES. Elemental analysis, Found: N, 2.309, C, 9.8, H 2.05. IR (KBr): ν = 3434 (vs; O–H), 2920 (w; C–H), 1636 (s; O–H), 1471 (w; –C=C–H), 1089 (vs; Si–O–Si), 798 (w; SiO–Si), 465 (m; Si–O).

2.5. Synthesis of SBA-RT

R6G-TETA (0.272 g, 0.5 mmol) and SBA-IPTES (1.0 g) were suspended in anhydrous toluene (50 mL) and stirred in reflux condition under N_2 for 24 h. The precipitate formed was filtered, and adequately washed several times with toluene and CH_2Cl_2 to rinse away any surplus R6G-TETA. A faint pink power was obtained and denoted as SBA-RT. Elemental analysis, Found: N, 2.878, C, 13.2, H 2.077. IR (KBr): ν = 3436 (vs; O–H), 2950 (w; C–H), 2830 (w; C–H), 1520 (s; –C=C–H), 1440 (w; –C=C–H), 1090 (vs; Si–O–Si), 809 (w; SiO–Si), 464 (m; Si–O).

2.6. Adsorption ability of SBA-RT for Cr(III)

SBA-RT (20 mg) and SBA-15 (20 mg) were added to the about 0.2 ppm Cr(III) solution (50 mL), respectively. The mixture was stirred for 4 h. After filtration, the concentration of residual Cr(III) in the filtrate was analyzed by inductively coupled plasma source mass spectrometer (ICP).

2.7. Imaging of HeLa cells incubated with SBA-RT and Cr(III)

HeLa cells were incubated with SBA-RT (1 ppm, suspended in PBS medium) for 30 min at 28 °C. After washing with saline to remove the remaining SBA-RT, HeLa cells were further incubated with 0.2 mM Cr(III) for another 30 min at 28 °C. HeLa cells were imaged by fluorescence microscopy.

2.8. Imaging of zebrafish incubated with SBA-RT and Cr(III)

Zebrafish was kept at 28 °C. For mating, male and female zebrafish was maintained in one tank at on a 12 h light/12 h dark cycle and then the spawning of eggs was triggered by giving light stimulation in the morning. Almost all the eggs were fertilized immediately. The 5-day old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH = 7.5). The 5-day old zebrafish was incubated with SBA-RT (4 ppm) in PBS media for 0.5 h at 28 °C. After washing with saline to remove the residual nanoparticles, the zebrafish was further incubated with 0.2 mM Cr(III) for another 30 min at 28 °C. Zebrafish was imaged by fluorescence microscopy.

3. Results and discussion

3.1. Synthesis and characterization of SBA-RT

Compound R6G-TETA was prepared according to the reported procedures. Intermediate material SBA-IPTES was easily synthesized by condensation of 3-(triethoxysilyl)propylisocyanate with SBA-15 in toluene [42]. SBA-RT was obtained by coupling of R6G-TETA with SBA-IPTES in toluene (Scheme 1) and characterized by elemental analysis (EA), XRD, and several spectroscopic methods.

Fig. 1 displays the FTIR spectra of SBA-15 and SBA-RT, new bands centered at around 3000–2800 cm^{-1} and 1500–1400 cm^{-1} were attributed to the C–H stretching and the aromatic stretching vibrations of Rhodamine group attached onto SBA-15, respectively [43]. The content of incorporated R6G-TETA receptor was determined by thermogravimetric analysis (TGA). As shown in Fig. 2, a slight weight loss (about 3.1%) of SBA-IPTES in the temperature