Talanta 97 (2012) 456–461

Contents lists available at SciVerse ScienceDirect

Talanta

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

Novel chitosan-based fluorescent materials for the selective detection and adsorption of Fe^{3+} in water and consequent bio-imaging applications

Qingtao Meng, Weiping Su, Cheng He, Chunying Duan*

State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian High-Tech Industrial Zone, Dalian 116024, China

ARTICLE INFO

Article history:
Received 7 December 2011
Received in revised form
21 April 2012
Accepted 30 April 2012
Available online 1 June 2012

Keywords:
Chitosan
Fe^{3+}
Detection
Absorbent
Fluorescence imaging

ABSTRACT

A series of fluorescent materials were synthesized by modification of chitosan (CS) with 4-fluorescein-carboxaldehyde (Fluo) and N-methyl-carbazole-3-aldehyde (Cb). Both **L-CS-Fluo** and **L-CS-Cb** feature excellent water-solubility and exhibit highly selective fluorescence response to Fe^{3+} in environment and biological fields. The high-molecular weight chitosan-based materials: **H-CS-Fluo** and **H-CS-Cb** take on doubled absorptivity compared to free chitosan due to the introduction of fluorescence probes. These modified-probe chitosan materials could be regenerated by treating with EDTA. Meanwhile, monomer probes **AG-Fluo** and **AG-Cb** linked by D-glucosamine were also synthesized to explore the binding efficiency.

1. Introduction

Iron is the most abundant transition metal ion present in the earth's crust with average content of approximately 5% in soil, sediment and rocks [1]. It is also an essential trace element that plays significant roles in chemical and biological processes [2–5]. For example, Fe^{3+} provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain, and both its deficiency and excess can induce a variety of diseases [6–8]. The drinking water standard for the maximum allowable level of Fe^{3+} in China is no more than 0.3 ppm [9]. Therefore, detection of Fe^{3+} in environmental and biological fields has aroused wide concern. Traditional detection methods, such as absorption spectrometry (AAS), inductively coupled plasma emission spectrometry (ICP-ES) and total reflection X-ray fluorimetry (TXRF) offer good limits of detection and wide linear ranges, but are very expensive and do not easily lend themselves to miniaturization [10]. Fluorescence probe is one of the best choices due to its high sensitivity and simplicity which translates molecular recognition information into tangible fluorescence signals [11]. Particularly, fluorescence probes are convenient to image intravital heavy transition metal (HTM) ions by *in situ* method. Currently, enormous efforts have been devoted to the development of Fe^{3+}-specific fluorescence probes [12,13]. However, most of them exhibited poor water solubility or biocompatibility restricting their applications. Moreover, their use in related analytical techniques in the homogeneous phase is not suitable for the separation, removal and enrichment of HTM ions in environmental field [14–15]. In recent years, we and other researchers have explored a novel way to design and prepare improved sensing materials by modification of mesoporous silica with fluorescence probes. These fluorescent materials were not only able to recognize HTM ions with high selectivity and sensitivity by fluorimetric or colorimetric method, but also can remove HTM ions from water sample [16–18]. At present, the selection of appropriate carriers has been a hotspot of study in this field.

Chitosan is one of the most abundant naturally occurring amino-polysaccharides and has attracted attention because of its unique physiochemical characteristics and biological activities [19–20]. First, chitosan is a carbohydrate biopolymer derived from deacetylation of chitin, the main component of crustacean exoskeletons. Chitin's abundance is second only to cellulose among polysaccharides found on Earth [21]. Second, the high contents of amino and hydroxyl functional groups of chitosan were beneficial to covalently graft organic functional molecules [22]. Lastly, the outstanding biodegradability and low toxicity of chitosan make it a desirable carrier to build biomaterials. Accordingly, chitosan-based materials have been widely used in environment [23–25], drug delivery [26–27], optical devices [28–31] and biomedical fields [32].

Herein, we reported a series of fluorescent materials by modification of chitosan with fluorescence dyes. We envisioned that these fluorescent materials could be used as fluorescence

* Corresponding author. Tel.: +86 411 84986261.
E-mail address: cyduan@dlut.edu.cn (C. Duan).

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http://dx.doi.org/10.1016/j.talanta.2012.04.062

probes and adsorbent in environmental field and fluorescence imaging agent in biological field. Furthermore, related detection and adsorption processes could be monitored by fluorimetric or colorimetric response methods. Meanwhile, monomer probes **AG-Fluo** and **AG-Cb** linked by D-glucosamine were also synthesized to explore the bonding mechanism.

2. Experimental

2.1. Reagents and instruments

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. D-Glucosamine hydrochloride, Chitosan and fluorescein were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Stock solution (2×10^{-2} M) of the aqueous nitrate salts of Fe^{3+}, Hg^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Ag^{+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Li^{+}, K^{+}, Na^{+} were prepared for further experiments.

^{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 **H-CS-Fluo** and **H-CS-Cb** for Fe^{3+} in water was measured by Inductively Coupled Plasma Spectrometer (Perkin Elmer). Cells were imaged by Nikon eclipase TE2000-5 inverted fluorescence microscopy.

2.2. General procedures of spectra registration/spectrophotometric detection

Stock solutions of **AG-Fluo**, **AG-Cb**, **L-CS-Fluo** and **L-CS-Cb** were prepared in Tris-HCl buffer (0.02 M pH=7.2). The cationic solutions were all in water with a concentration of 2.0×10^{-2} M for spectrometric analysis. Excitation wavelength for **AG-Fluo** and **L-CS-Fluo** was 470 nm. Excitation wavelength for **AG-Cb** and **L-CS-Cb** was 350 nm for the fluorescence titration of Fe^{3+}. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to corresponding solution. Each time a 2 mL solution of probe was filled in a quartz cell of 3 cm optical path length, and different stock solutions of cations were added into the quartz cell gradually by using a micro-syringe. The volume of cationic stock solution added was less than 100 μL with the purpose of keeping the total volume of testing solution without obvious change.

2.3. Synthesis and characterization the fluorescent materials

2.3.1. Synthesis of 4-fluoresceincarboxaldehyde and N-methyl-carbazole-3-aldehyde

4-fluoresceincarboxaldehyde and N-methyl-carbazole-3-aldehyde were prepared according to reported procedures [33–34].

2.3.2. Synthesis of ***AG-Fluo***

30 mL methanol solution of 4-fluoresceincarboxaldehyde (0.99 g, 2 mmol) was added to the solution D-glucosamine·HCl (0.43 g, 2 mmol) and triethylamine (0.3 mL). The mixture was refluxed for 4 h 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-FLuo** as a yellow solid in 45% yield. Elemental analysis: calcd (%) for $C_{27}H_{23}NO_{10}$: C 62.19, H 4.45, N 2.69%. Found (%): C 62.21H 4.461N 2.716%. ESI-MS negative peak at m/z=522.0 indicated [**AG-Fluo**+H]$^{+}$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm):10.30 (s, 1H), 9.08 (s, 1H), 8.07 (s, 1H), 7.86 (d, 1H), 7.79 (d, 1H), 7.34 (d, 1H), 7.04 (m, 1H), 6.88 (d, 1H), 6.72 (d, 1H), 6.61 (d, 1H), 6.38 (d, 1H), 5.47 (m, 1H), 5.42 (m, 1H), 5.33 (d, 1H), 5.18 (d, 1H), 4.86 (d, 1H), 4.63 (t, 1H), 3.84 (t, 1H), 3.73 (m, 1H), 3.65 (m, 2H), 3.55 (m, 1H), 3.33 (m, 1H), 3.25 (t, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 61.48, 67.74, 70.78, 71.61, 72.96, 74.83, 77.37, 91.22, 95.35, 102.89, 104.28, 110.16, 113.78, 115.78, 119.42, 124.52, 125.14, 129.49, 130.53, 133.05, 134.03, 136.10, 151.61, 160.82, 161.75, 169.22. IR (KBr): 3422 cm^{-1}, 2936 cm^{-1}, 1730 cm^{-1}, 1649 cm^{-1}, 1585 cm^{-1}, 1527 cm^{-1}, 1471 cm^{-1}, 1373 cm^{-1}, 1293 cm^{-1}, 1171 cm^{-1}, 1099 cm^{-1}, 1042 cm^{-1} and 581 cm^{-1}.

2.3.3. Synthesis of ***L-CS-Fluo***

4-fluoresceincarboxaldehyde (0.99 g, 2 mmol) was dissolved in 50 mL methanol. Then water-soluble low-molecular weight chitosan (L-CS) (2.0 g) was added. The mixture was refluxed for 8 h under N_2. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting powder was dissolved in 20 mL methanol and washed with CH_2Cl_2/CH_3OH (1:15, V/V) on a silica gel chromatography to wash off the nonbounded 4-fluorescein-carboxaldehyde. Methanol was then added constantly until the yellow production drain away. The collected solution was evaporated in vacuum to afford yellow solid **L-CS-Fluo** in 48% yield. Elemental analysis: found (%): C 34.10, H 5.506, N 3.996. IR (KBr): 3442 cm^{-1}, 3006 cm^{-1}, 2920 cm^{-1}, 2744 cm^{-1}, 1629 cm^{-1}, 1520 cm^{-1}, 1383 cm^{-1}, 1290 cm^{-1}, 1245 cm^{-1}, 1206 cm^{-1}, 1140 cm^{-1}, 1080 cm^{-1}, 1022 cm^{-1}, 924 cm^{-1}, 812 cm^{-1}, 741 cm^{-1}, 573 cm^{-1} and 453 cm^{-1}.

2.3.4. Synthesis of ***H-CS-Fluo***

4-fluoresceincarboxaldehyde (0.99 g, 2 mmol) was dissolved in 50 mL methanol. Then high-molecular weight chitosan (H-CS) (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 Sechelt's extractor and extracted with methanol for at least 12 h to ensure that there was noncovalently bounded 4-fluoresceincarboxaldehyde in chitosan. After drying under reduced pressure, the reaction afforded **H-CS-Fluo** as yellow solid. Elemental analysis: found (%):C 43.45, H 6.661, N 7.085. IR (KBr): 3440 cm^{-1}, 2990 cm^{-1}, 2883 cm^{-1}, 1755 cm^{-1}, 1600 cm^{-1}, 1469 cm^{-1}, 1280 cm^{-1}, 1051 cm^{-1} and 463 cm^{-1}.

2.3.5. Synthesis of ***AG-Cb***

4-fluoresceincarboxaldehyde (0.99 g, 2 mmol), D-glucosamine·HCl (0.43 g, 2 mmol) and triethylamine (0.3 mL) were mixed in 30 mL methanol. After the solution was refluxed for 3 h with stirring, pale precipitates obtained were filtered, washed with cold methanol (3×5 mL) and dried under vacuum with 85% yield. Elemental analysis: calcd (%) for $C_{20}H_{22}N_2O_5$: C 64.85, H 5.99, N 7.56%. Found (%): C 64.24, H 5.95 and N 7.15%. ESI-MS positive peak at m/z 371.0 indicated [**AG-Cb**+H]$^{+}$. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.51 (s, 1H), 8.34(s, 1H), 8.23 (s, 1H), 7.90 (d, 1H), 7.63 (d, 1H), 7.50 (t, 1H), 7.25 (t, 1H), 6.55 (d, 1H), 4.92 (d, 1H), 4.83 (d, 1H), 4.76 (t, 1H), 4.55 (t, 1H), 3.91 (s, 3H), 3.76 (m,