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A novel chromogenic molecular sensing platform for highly sensitive and selective detection of Cu^{2+} ions in aqueous environment

Mecit Ozdemir

Department of Food Processing, Vocational School of Technical Sciences, Kilis 7 Aralik University, Kilis, Turkey

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ABSTRACT

A new chelate-type of FBO probe based on a spirocycle fluorescein hydrazone derivative was developed for the rapid, ultrasensitive, and highly selective detection of Cu^{2+} ions in aqueous media. Sensor FBO and its copper complex were characterized by elemental, magnetic moment and TGA-DTA analyses, FT-IR, ^1H NMR and ^{13}C NMR spectroscopies, and Tandem MS spectrometry. Probe FBO showed excellent selectivity and sensitivity only for Cu^{2+} among other biologically and environmentally important metal ions in EtOH-H_2O (2:1, v/v, 20 mM Tris-HCl, pH 7.4). The binding interaction of FBO to the Cu^{2+} ion resulted in the spirolactam ring-opening mechanism associated with the colorimetric 'turn-on' response in the UV–vis absorption spectra above 350 nm. This molecular sensing platform established a chromogenic method for quantification and visualization of Cu^{2+} ions with the naked eye by forming a 1:1 receptor-copper complex under physiological pH conditions. Furthermore, the detection limit of FBO for Cu^{2+} was determined to be as low as 7.4 nM, which is much lower than the limits set by the U.S. EPA and WHO. More importantly, chemosensor FBO was successfully applied for the direct detection of Cu^{2+} ions in aqueous media.

1. Introduction

After iron and zinc, copper is one of the most abundant transition metals and a crucial micronutrient for animals and plants. In particular, it plays an essential role in the proper function of organisms' immune systems due to its redox activity as a cofactor in many metalloenzymes, including cytochrome c oxidase (respiration), copper amine oxidases (for handling of dietary amines), Cu/Zn superoxide dismutase (SOD1), ceruloplasmin (for electron transfer/substrate oxidation and iron uptake), tyrosinase (for pigmentation), hephaestin (for the metabolism and homeostasis of iron and copper), lysyl oxidase (for the synthesis of collagen and elastin), dopamine β-hydroxylase and peptidylglycine monooxygenase (for neurotransmitter synthesis and metabolism). As a redox-active nutrient, excessively accumulated copper in the human body from copper-contaminated water leads to various genetic diseases, including Alzheimer's, Parkinson's, Menkes, Prion, Huntington's and Wilson's diseases. In contrast, however, a deficiency of copper in the human body can increase the risk of coronary heart disease due to it being an essential trace nutrient for all organisms [1–5]. Aside from these concerns, it is extensively used in industrial sectors, such as in the making of alloys, electrical wires, machine parts, drugs, fertilizers, and batteries, as well as in waste combustion and steel processing. These industrial activities are linked with soil and water contamination and copper uptake by plants. In recent years, due to its excessive loading and participation in the formation of reactive oxygen species (ROS), the toxicity of copper has become an important issue for all living organisms around the world. The effective detection and monitoring of heavy and transition metal ions (HTMs), especially copper ions, in food, drinking water, industrial, and biological samples is therefore an important topic for future research [1,6,7].

To the best of our knowledge, several common analytical technologies—such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), electrochemical methods, surface plasmon resonance (SPR) techniques, and quantum-dot (QD)-based sensors—have been established for the qualitative and quantitative detection of copper ions at trace levels [7,8]. Despite the success of the commonly used traditional methods, however, these techniques generally require expensive and sophisticated instrumentation, complex sample pretreatment, a time-consuming process, and operational management challenges. They also need specific equipment and expert staff. In light of this, colorimetric and fluorescent molecular-sensing platforms that are capable of binding to toxic metal ions have recently attracted considerable attention from scientists and researchers due to their operational simplicity, low cost, real-time analysis, fast response, high

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E-mail address: mecitozdemir@kilis.edu.tr.

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reliability, and selectivity and sensitivity for heavy and transition metal ions in aqueous media [9–13].

Over the last two decades, among the colorimetric and fluorescent chemosensors, the alternative analytical approaches of spirocycle fluorescein and rhodamine-derived fluorophores have attracted a great deal of research interest in the fields of analytical chemistry, biochemistry and environmental science. This has been mostly due to their excellent photophysical properties, such as the high fluorescence quantum yield, good photostability, simplicity, a low detection limit, capability for extraordinary recognition, high absorption coefficient, and broad fluorescence in the visible region of the electromagnetic spectrum. So far, many highly valuable chemosensors have been designed and developed as selective and sensitive signaling platforms for the determination and visualization of the most commonly used heavy and transition metal ions (HTMs) in several environmental, chemical and biological applications [14–22]. In addition to other outstanding features, the chemosensors based on spiroring-closed of xanthenes and related derivatives are generally colorless and non-fluorescent. Upon treatment with the cations of various metals, however, the open-cyclic forms of the sensor structures induced by metal ions lead to not just strong absorbance and/or fluorescence intensity changes. They also trigger a distinct color change from colorless to a color—such as deep yellow, pink, or purple—which then allows for direct detection of targeted ions with the naked eye [23–30]. In view of the marked points, colorimetric and/or fluorescent chemosensors based on the rhodamine and fluorescein frameworks have remarkable advantages that facilitate the rapid detection and real-time monitoring of different chemical species in biological, environmental, and industrial samples.

In this contribution, a new colorimetric 'turn-on' sensor FBO, consisting of fluorescein hydrazide linked to 2-acetylpyridine, was developed for the ultrasensitive quantitation of copper ions in aqueous media. To evaluate the selectivity, competitive ability, and reversible potential of the present molecular platform FBO to Cu^{2+} ions, UV–vis titration studies were conducted in EtOH-H_2O (2:1, v/v, 20 mM Tris-HCl, pH 7.4). Upon treatment with Cu^{2+}, the color of the solution containing probe FBO changed from a colorless state to deep yellow, and this was accompanied by broadened absorption bands above 350 nm. However, the addition of excessive amounts of the other sixteen competitive metal ions did not cause any changes either in color or UV–vis absorption spectra. The experimental results demonstrate that the intrinsic chromogenic probe FBO could be applied as a promising signal switcher, thus making possible the 'naked-eye' detection of Cu^{2+} ions with a very low detection limit in aqueous environments.

2. Experimental details

2.1. Instruments

The structural characterization of FBO and its copper complex was carried out using FT-IR, ^1H NMR, ^{13}C NMR, LC–MS/MS, elemental analyses, fluorescence spectra, and UV–vis spectra methods. ^1H NMR and ^{13}C NMR spectra were recorded on an Agilent Technologies NMR system 400 and Bruker AVANCE III HD 600 MHz NMR spectrometer. These spectra were collected in deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal standard. All tandem mass spectra were acquired on a Thermo Scientific™ TSQ Quantum™ Access MAX Triple Quadrupole Mass Spectrometer. Elemental microanalysis for carbon, hydrogen, and nitrogen (C, H, & N) was carried out using a LECO Truspec Micro CHNS microanalysis apparatus. FT-IR spectra, meanwhile, were recorded over the 400–4000 cm^{-1} range using a PerkinElmer Spectrum 100 spectrometer or Bruker ALPHA infrared spectrometer. Thermogravimetric (TG) and differential thermal (DT) analyses of FBO-Cu^{2+} complex were conducted using a Shimadzu DTG-60H thermal analyser in the 20–1000 °C range by heating a sample of about 9.71 mg at a constant rate of 10 °C per minute under a nitrogen atmosphere. The magnetic moment for the FBO-Cu^{2+} complex was measured using a Sherwood MSB balance according to Gouy's method at room temperature (298 ± 1 K). Copper content in tap water samples was quantified through atomic absorption spectroscopy (AAS) using an Agilent Technologies 200 Series AA with a GTA 120 graphite tube atomizer. The fluorescence spectra were measured using quartz cells, a 10 mm pathway length, on a Hitachi F-7000 fluorescence spectrophotometer at 298 ± 1 K. UV–vis absorption spectra at room temperature (298 ± 1 K) were recorded on a Thermo Scientific Multiskan GO, while the melting point was carried out in an open capillary tube using the Stuart SMP30 melting point apparatus. A Voltmeter 0–3/15 V Da was used in the setup of a voltaic cell that generated direct current from the chemical reaction. All pH measurements were carried out using a multiparameter analyser (Consort C5020) that was calibrated with a standard buffer solution.

2.2. Chemicals

Unless otherwise indicated, it was intended that all chemicals were purchased from commercial suppliers. All reagents were of an analytical grade and used without further purification. Distilled water was used throughout the UV–vis absorption experiments. Fluorescein hydrazide was synthesized according to the method reported in previous works [4,10]. To monitor the progress of the reaction, thin layer chromatography (TLC) was used on silica gel plates. For the purification process, silica gel column chromatography was performed with dichloromethane (DCM) and methanol (MeOH) as the mobile phase. Ethylenediaminetetraacetic acid (EDTA) was used as a chelating agent in the reversibility assay. A stock solution of probe FBO (1 mmol.L^{1-}) was prepared by dissolving 6.75 mg of sensor in 15 ml of ethanol, which was very stable for several months. The nitrate or chloride salts—Na^+, K^+, Ca^{2+}, Mg^{2+}, Cr^{3+}, Fe^{3+}, Hg^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Al^{3+}, Mn^{2+} and Ag^+—were dissolved in distilled water to create 1 mmol.L^{1-} aqueous solutions. All stock solutions were kept in a refrigerator at 4–6 °C.

2.3. UV–vis Absorption measurements

A standard test solution at 1.0×10^{-3} molar (M) of receptor FBO in ethanol prepared was then diluted to obtain micromolar (μM) concentrations by using EtOH-H_2O (2:1, v/v, 20 mM Tris-HCl, pH 7.4) for the UV–vis absorption titration experiments. All measurements were conducted under identical conditions at room temperature (298 ± 1 K). All titration experiments were performed by gradually adding a corresponding concentration of metal ions into the FBO solution. All absorption spectra were measured in solutions using quartz cuvettes, a 1 cm pathway length, and a wavelength range from 300 to 600 nm (every 1 s). Upon addition of each relevant metal species to the FBO solution, the spectral readings were immediately recorded at very short intervals.

2.4. Application of probe FBO to the quantification of Cu^{2+} in tap water samples

In order to explore the suitability and feasibility of probe FBO, it was first used to detect Cu^{2+} ions in tap water samples collected in K.Maras, Turkey. Initially, the tap water samples were spiked with standard solutions of Cu^{2+} and diluted with EtOH to derive samples at concentrations of 0, 0.5, 2, and 4 mg. L^{-1} (ppm) Cu^{2+}, respectively. Subsequently, the absorption measurements were carried out by using the proposed detection system under the same experimental conditions. For the purpose of checking and providing a comparison, the AAS method was further employed to quantitatively determine the trace Cu^{2+} ions in the spiked water.