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A highly selective dual-channel Cu^{2+} and Al^{3+} chemodosimeter in aqueous systems: Sensing in living cells and microfluidic flows

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ABSTRACT

The design and development of fluorescent chemosensors have recently been the focus of considerable attention for the sensitive and specific detection of environmentally and biologically relevant metal ions in aqueous solutions and in living cells. Herein, we report photophysical results for a 1*H*-pyrrole-2-carboxaldehyde-substituted rhodamine 6G derivative (RCS) that possesses specific binding affinity toward Al^{3+} and Cu^{2+} at micromolar concentration levels. In an *N*,*N*-dimethylformamide (DMF) and water (v/v = 2/8) medium, the RCS chemosensor exhibits a substantially enhanced absorbance intensity at 532 nm and a color change from colorless to pink for Cu^{2+}; it also exhibits significant "off–on" fluorescence at 557 nm, accompanied by a color change from colorless to fluorescent-yellow upon binding to Al^{3+}. The RCS sensor exhibits extremely high fluorescence enhancement upon complexation with Al^{3+}, and it can be used as a "naked eye" sensor. Through fluorescence titration at 557 nm, we confirmed that RCS exhibits a fluorescence response with a remarkable enhancement in emission intensity resulting from the complexation between RCS and Al^{3+}, whereas no emission appeared in the case of competitive metal ions (Cu^{2+}, Al^{3+}, Li^{+}, Na^{+}, K^{+}, Cs^{+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Co^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+}) in a DMF and water (v/v = 2/8) solution. The reversible ring-opening mechanism of the rhodamine spirolactam induced by Al^{3+}/Cu^{2+} binding and the 1:1 stoichiometric structure between RCS and Al^{3+} were adequately supported by Job-plot evaluation, optical titration and FT-IR analysis. The lowest detection limit for Al^{3+} is $3.20 \times 10^{6}\ M^{-1}$ in a DMF and water (v/v = 2/8) solution. Al^{3+}-induced chelation-enhanced fluorescence (CHEF) is associated with spirolactam ring opening of the rhodamine unit. Finally, RCS was successfully applied for the bio-imaging of Al^{3+} in living HeLa cells and for fluorescence imaging of a microfluidic system.

1. Introduction

Extraordinary progress has been made in the design and synthesis of fluorescent chemosensors based on various platforms over the past several years. Recently, interest in colorimetric and fluorescence chemosensors has been increasing because of their potential biological and environmental applications [1–4]. Thus, various metal cations have been the primary targets of chemosensor researchers because of their harmful effects to the ecosystem [5–10]. Among these metal cations, copper (Cu^{2+}) and aluminum (Al^{3+}) ions have been widely used as specific analytes during investigations of chemosensors. Among the essential heavy-metal ions in the human body, Cu^{2+} is the third most abundant after Fe^{3+} and Zn^{2+}, and Cu^{2+} plays very important roles in several biological processes [11–14]. Copper, together with certain proteins, can also produce numerous enzymes that are essential for life, such as cytochrome c oxidase, tyrosinase and superoxide dismutase [15–18]. A deficiency of Cu^{2+} leads to Menke's disease, which is characterized by sparse and coarse hair, growth failure and deterioration of the nervous system. The U.S. Environmental Protection Agency (EPA) has set the maximum allowable level of copper in drinking water at 1.3 ppm [19]. In addition, Cu^{2+} is also used to treat plant diseases, as a colorant for china and as a preservative for wood and leather in industry. The potential toxic effects of Cu^{2+} on human beings continue to be challenging problems worldwide. Exposure to high levels of Cu^{2+} leads to Wilson's disease,

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gastrointestinal disorders and kidney damage. A high level of copper has also been suggested to cause serious diseases such as Alzheimer's disease and prion disease [20,21]. In this regard, copper can contribute to redox stress and lead to impairment. Therefore, copper is related to neurodegenerative diseases such as Wilson's disease and Menke's disease [22]. Consequently, the effective detection of Cu^{2+} in water or physiological samples is of toxicological and environmental importance [23–25]. Copper ions can be detected using several instrumental techniques [26–32]. However, these methods are time consuming and require expensive instrumentation.

Aluminum is the most abundant (8.3% by weight) metallic element and the third-most abundant element (after oxygen and silicon) on Earth. The widespread application of Al^{3+} in modern society includes its use in water treatment, food additives, medicines and the production of light alloys, which often expose people to aluminum ions [33,34]. Aluminum is also toxic to humans, fish, and plants through acid rain, and it affects other biological processes as a result of human activities [35]. Osteoporosis is another disease related to the presence of aluminum [36]. A more dangerous disease, Alzheimer's disease (a degenerative brain disease), has been linked to both copper and aluminum metal ions [22,35,36]. Aluminum accumulation has been shown to cause lung, breast, and bladder cancer [37,38]. Aluminum may also directly affect the metabolism of iron by influencing its absorption via the intestine, by hindering its transport in serum, and by displacing it by binding to transferrin [39]. Furthermore, nearly 40% of acidic soils worldwide are thought to be polluted because of the effects of aluminum toxicity, which is the critical factor that hinders crop production in acidic soils [40–42]. For these reasons, the detection of Al^{3+} is critical for controlling its levels in the environment and for minimizing the direct impact of aluminum on human health.

Among the various detection techniques, optical detections (via fluorescence or colorimetric changes) are the most promising methods because of their simplicity, low detection limits and useful applications in environmental chemistry, medicine and biology [2,43–48]. Among these methods, the most important advantage of a fluorescent probe is intracellular detection. Considerable efforts have recently been devoted to the development of fluorescent and colorimetric sensors for the selective determination of metal ions [49]. Fluorophores are specific resources that provide molecular recognition signals based on changes in color, absorption and fluorescence. Because of their excellent optical properties, many types of fluorophores have been explored for use in chemosensor materials [50]. Among these fluorophores, rhodamines have been reported by many investigators to be a strong fluorophore for chemosensitivity because of their excellent photostabilities and photophysical properties [51]. However, rhodamine derivatives and its ring-opening reactions have received considerable attention from organic chemists only since 1997 [52]. Spirocyclic forms of rhodamine derivatives are useful sensing platforms because the ring-opening process contributes to a fluorescence change and gives rise to strong fluorescence and a pink color [53]. Furthermore, rhodamine has a long fluorescence wavelength (greater than 550 nm), which is frequently favored as a reporting group for analytes to avoid the influence of background fluorescence at wavelengths shorter less than 500 nm [54].

The development of application tools that employ a chemosensor, such as bio-imaging [55–59] and microfluidic systems [60–63], has recently gained increasing attention. The use of chemosensors in bio-imaging applications is important for monitoring and efficiently detecting various types of metal ions in living cells of the human body. A microfluidic system combined with a chemosensor can be a device of considerable importance in the development of lab-on-a-chip systems [64,65]. As an analysis tool, this system can offer various benefits, such as a low fluid volume consumption and simple parallelization [66,67].

Our work in this area has focused on optical methods and has explored a number of chemosensor systems based on the concept of intramolecular charge transfer (ICT) [68,69]. In our previous studies, we investigated the chemosensitivity of rhodamine and squaraine derivatives [70–74]. In this work, we report the design and synthesis of a novel rhodamine-based chemosensor (RCS) through the reaction of rhodamine 6G hydrazide and 1*H*-pyrrole-2-carboxaldehyde for the detection of metal ions in aqueous media. In this respect, the RCS was investigated and observed to exhibit highly selective colorimetric and fluorescence sensing abilities for Cu^{2+} and Al^{3+}, respectively. The optical properties were investigated using UV-Vis and fluorescence analyses in aqueous media to determine the fluorescence response of RCS for Al^{3+} for various applications, such as bio-imaging and microfluidic systems.

2. Experimental

2.1. Materials, equipment for the synthesis of RCS and optical analysis

All materials used for the synthesis of RCS were purchased from Aldrich and Alfa Aesar and were used without further purification. All of the inorganic salts used to analyze the sample were purchased from Aldrich and Alfa Aesar in the form of perchlorate salts. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL-AL400 (Japan) operating at 400 MHz and on a BRUKER AVANCE III 600 (Germany) operating at 600 MHz. Electrospray ionization mass spectrometry (ESI-MS) was conducted using a Jeol MStation [JMS-700] mass spectrometer. The melting point was determined using a Barnstead Electrothermal A9200 apparatus. High-resolution mass spectra (HRMS) were recorded on a micrOTOF-QII (Bruker, Daltonik, Germany) mass spectrometer. Absorption and fluorescence spectra were recorded using an Agilent 8453 spectrophotometer and a Shimadzu RF-5301PC fluorescent spectrophotometer, respectively. *N*,*N*-Dimethylformamide (DMF) and deionized water were used as solvents. Deionized and distilled water were purified using a New Human RO 180. pH was measured with a Mettler Toledo MP220 digital pH meter.

2.2. Synthesis of RCS

Rhodamine 6G hydrazide was prepared according to previously reported methods [75–77]. Rhodamine 6G hydrazide (1.0 mmol, 0.428 g) and 1*H*-pyrrole-2-carboxaldehyde (1.0 mmol, 0.095 g) were mixed in boiling methanol with a few drops of acetic acid under a nitrogen atmosphere. After the reaction mixture was refluxed for 4 h, a white precipitate was collected by filtration and the obtained precipitate was washed with methanol/ether (1:1, v/v). The product was purified via column chromatography using silica gel and CH_3OH:CH_2Cl_2 (1:10, v/v). The obtained yield was 0.2964 g (59.0%). M.P. = 312 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.00 (s, 1H), 8.06 (s, 1H), 7.965 (q, 1H), 7.44 (q, 1H), 7.26 (s, 1H), 7.01 (q, 1H), 6.79 (s, 1H), 6.38 (s, 2H), 6.32 (s, 2H), 6.20 (s, 1H), 6.08 (q, 1H), 3.49 (s, 2H), 3.20 (q, 4H, J = 7.1 Hz), 1.88 (s, 6H), 1.31 (t, 6H, J = 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 165.35, 152.67 (C=O), 151.0, 147.6, 137.8, 133.3, 128.6, 128.3, 128.2, 127.6, 123.5, 123.2, 121.5, 118.2, 114.0, 109.3, 105.8, 96.8, 65.7 (Spiro-carbon), 38.4, 16.7, 14.8; ESI-MS [m/z] = 506.2 $[M+H]^+$, calculated for $C_{31}H_{31}N_5O_2$ = 505.2. HRMS (ESI) calcd for $C_{31}H_{31}N_5NaO_2$ ($[M+Na]^+$): 528.24, found: 528.2380.

2.3. Bio-imaging study

For the cell viability study, the cytotoxicity of the synthesized chemosensor was analyzed using an MTT assay. HeLa cells