A ratiometric Al^{3+} ion probe based on the coumarin-quinoline FRET system

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ABSTRACT

A coumarin-quinoline based fluorescence resonance energy transfer (FRET) system (**TCQ**) has been synthesized and employed as a ratiometric fluorescence probe. The selective fluorescent response of the probe **TCQ** toward Al^{3+} was devised by employing a quinoline moiety as a FRET energy donor with a coumarin moiety as an energy acceptor. The quinoline emission at 390 nm decreased and the coumarin emission at 480 nm increased concurrently on addition of Al^{3+} under excitation wavelength at 253 nm. The **TCQ** probe exhibited high selectivity for Al^{3+} as compared to other tested metal ions and the ratiometric sensing of Al^{3+} was determined by plotting the fluorescence intensity ratio at 480 nm and 390 nm versus Al^{3+} ion concentration. Moreover, test strips based on **TCQ** were fabricated, which were found to act as a convenient and efficient Al^{3+} ion detection kit. Furthermore, this system has been used for imaging of Al^{3+} in living cells.

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1. Introduction

Many fluorescent probes have been synthesized and subsequently employed for biosensing, bioimaging, and in environmental detection. This widespread use is due to a number of desirable features such as real time monitoring of fluorescence with high selectivity and sensitivity, non-destructive analysis and simple instrumentation [1]. As we all known, metal ions are ubiquitous and play a fundamental role in a wide range of chemical and biological processes. Undoubtedly, the understanding of metal ion homeostasis in biology has significantly benefited from advancements in the development of fluorescent probes for monitoring metal ions. Aluminum is the third most prevalent element and the most abundant metal in the earth's crust, and has been widely used in many fields [2]. Excess levels of Al^{3+} in the human body can cause adverse physiological effects and leads to lots of diseases such as microcytic hypochromic anemia, bone softening, encephalopathy, myopathy and Alzheimer's disease [3]. So, highly sensitive and selective bioimaging of Al^{3+} in the cell is required to understand the underlying mechanism about how aluminum ions cause aluminum-induced human diseases. Thus, detection of

Al^{3+} is very significant to monitor the concentration level in the environment and minimize direct effect of the Al^{3+} ion on human health [4]. Numerous probes for Al^{3+} have been reported which displayed a decrease or increase in the emission intensity [5–18]. Most of them are fluorescence intensity-based probes, which mean that ion detection depends on a simple change of fluorescence intensity arising from metal-binding, and tends to be significantly influenced by the excitation power, detector sensitivity and instrument environmental factors, especially at very low ions concentrations. To alleviate the above problems, ratiometric measurements were developed for precise and quantitative analysis of biological events occurring under complex conditions by simultaneously recording fluorescence intensities at two wavelengths and then calculating their ratios [19]. However, despite many potential advantages, examples of ratiometric fluorescence probes for Al^{3+} [20,21], $Fe^{3+}/Al^{3+}/Cr^{3+}$ [22], Al^{3+}/Zn^{2+} [23,24], and Al^{3+}/Hg^{2+} [25] are scant.

The application of fluorescence resonance energy transfer (FRET) should be an efficient approach to the design of ratiometric fluorescence probes, since they can emit at two different wavelengths at a single excitation source. FRET is a distance-dependent interaction between the electronic excited states of two different fluorescent groups in which excitation is transferred from a donor moiety to an acceptor moiety without photoemission. The efficiency of FRET is primarily controlled by the scope of spectral

overlap between donor emission with acceptor absorption and the distance between donor and acceptor [26,27]. Because the pseudo Stokes shifts of FRET based probes are larger than the Stokes shifts of either the donor or acceptor dyes, the possibility of self-quenching as well as fluorescence detection errors due to backscattering effects of the excitation source will be efficiently avoided [28].

Given the high flexibility in the design of FRET probes and the wide choice of fluorophore available, FRET has been widely utilized for the design of ratiometric probes, such as for pH [29], Fe^{3+} [30], Cu^{2+} [31,32], Hg^{2+} [33], H_2S and sulfide/sulfite [34,35].

Notably, so far only a few FRET-based probes for the detection of Al^{3+} have been reported [36–38]. These probes have demonstrated some promising attributes such as high specificity and sensitivity; however, there are still a number of limitations and most of them are based on rhodamine. Therefore the development of new ratiometric FRET-based probes for Al^{3+} ions with improved detection limits in biological systems are desirable.

The important challenge is selectivity of suitable "Donor-Acceptor" pairs, because a requirement for Forster energy transfer is that the emission spectrum of the donor must overlap with the absorption spectrum of the acceptor [39]. Owing to their excellent photochemical and photophysical properties, rhodamine and coumarin derivatives have often been used to construct FRET-based probes for detecting different species, typically $Hg^{2+}/Cu^{2+}/Fe^{2+}/Fe^{3+}$ [40], Cu^{2+} [41], H_2S [42–44], pH [45], Fe^{3+} [46], Cu^{2+}/Hg^{2+} [47,48], HOCl [49].

Coumarin and quinoline dyes have been widely used as fluorophores owing to their favourable biocompatibility and photophysical properties [50,51]. In addition, coumarin dyes, for which the emission profiles are tunable from the blue to near-infrared region by simply modifying the coumarin core structure, have been extensively applied in fluorescent probes [52]. Moreover, the emission spectrum of coumarin and the excitation spectrum of quinaldine have substantial overlap, which would fulfill an important requirement for the FRET process.

Herein, we report the design, synthesis, and application of a FRET-based ratiometric Al^{3+} probe (**TCQ**) by combining the coumarin fluorophore in a quinoline derivative using a triazole linker. In this sensing system, the quinoline moiety was chosen as the energy donor, since its fluorescence spectra can match well with the absorption spectra of coumarin (the energy acceptor). Furthermore, fluorescence imaging by **TCQ** for Al^{3+} in living cells was demonstrated.

2. Experimental

2.1. Materials and equipments

Deionized water was used throughout the experiments. Other chemicals were purchased from Alfa Aesar Co., Tianjin, China and used without further purification. The solutions of metal ions were prepared from their nitrate salts which were analytical grade.

Fluorescence spectra measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Varian) equipped with a xenon discharge lamp using a 1 cm quartz cell. UV–vis spectra were recorded on a UV-1800 spectrophotometer (Beijing General Instrument Co., China). IR spectra were obtained using a Vertex 70 FT-IR spectrometer (Bruker). Melting points were determined on an X-5 binocular microscope (Beijing Tech Instrument Co., China). ^1H and ^{13}C NMR spectra were measured on a Nova-400 NMR (Varian) and WNMR-I 500 NMR spectrometer (at the Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences) using TMS as an internal standard. ESI–MS spectra was recorded on a HPLC–MSD-Trap-VL spectrometer (Agilent) and MALDI-TOF mass spectra was collected on a BIFLEX III ultra-high

resolution Fourier transform ion cyclotron resonance mass spectrometer (Bruker) with α-cyano-4-hydroxycinnamic acid as matrix. ITC experiments were performed using a Nano isothermal titration calorimeter (TA). The cell imaging test was carried out with an eclipse Ti-U (Nikon, Japan) inverted fluorescence microscopy.

2.2. Solution preparation

The **TCQ** stock solution (1 mM) was prepared using a 100 mL volumetric flask, with 43 mg of **TCQ** dissolved in EtOH and then diluting to the mark.

The Al^{3+} stock solutions (2 mM) were prepared in a 50 mL volumetric flask by dissolving 40.0 mg $Al(NO_3)_3$ in H_2O, and then diluting to the mark with H_2O. The standard stock solution of other metal ions (2 mM) Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, Fe^{3+}, Cr^{3+}, Ga^{3+}, In^{3+} and As^{3+} were prepared by dissolving an appropriate amount of their perchlorate or nitrate in water and adjusting the volume to 100 mL in a volumetric flask.

The Tris-HCl buffer stock solution was prepared in water solutions (1 mM, pH 7) with the requisite amount of HCl.

The ^1H NMR spectroscopic experiments used analytical grade Al $(ClO_4)_3$.

The ITC experiment consisted of 25 consecutive injections (10 μL) of Al^{3+} ion solution (1 mM) into the microcalorimetric reaction cell (1 mL) charged with a solution of **TCQ** (0.1 mM). The heat of reaction was corrected for the heat of dilution of the ion solution determined in the separate experiments. All solutions were degassed prior to the titration experiment by sonication. Computer simulations (curve fitting) were performed using the Nano ITC ananlyze software.

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) and PC3 cells were grown in Roswell Park Memorial Institute-1640 (RPMI-1640), respectively, supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C and 5% CO_2. One day before imaging, the cells were seeded in 6-well flat-bottomed plates. The next day, cells were incubated with 10 μM **TCQ** for 60 min at 37 °C in a humidified environment of 5% CO_2 and then washed with fresh culture medium three times to remove the remaining **TCQ**. Before incubating with 50 μM Al^{3+} for another 50 min, cells were rinsed with fresh culture medium three times again, then the fluorescence imaging of intracellular Al^{3+} was observed using an inverted fluorescence microscope (excited with UV light). Cells which were only incubated with 10 μM **TCQ** for 60 min at 37 °C under 5% CO_2 were used as a blank control.

2.3. Synthesis of probe **TCQ**

The synthetic route of **TCQ** was carried out as outlined in Scheme 1:

To a solution of 8-hydroxyquinaldine (1.00 g, 6.29 mM) in acetone solution (50 mL) was added K_2CO_3 (1.30 g, 9.44 mM). The mixture was heated under reflux for 0.5 h. After cooling, 3-bromopropyne (3.70 g, 31.45 mM) was added, and then the mixture was heated at reflux for 18 h. The reaction mixture was filtered and the solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (v/v, 4/1) as the eluent to give the target intermediate compound (1.05 g, 5.33 mM) in 85.0% yield.

A solution of the intermediate compound (1.00 g, 5.08 mM), 7-(2-azido-ethoxy)- coumarin (2.34 g, 10.15 mM) and CuI (catalytic amount) was reacted in THF/H_2O (v/v, 10/1) at room temperature for 10 h. The crude product was purified by column chromaography on silica gel using petroleum ether/ethyl acetate (v/v, 1/1) as the