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A silica nanoparticle-based dual-responsive ratiometric probe for visualizing hypochlorite and temperature with distinct fluorescence signals

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ABSTRACT

Hypochlorite (ClO^-) and temperature play crucial roles in a wide range of physiological processes, and they are also implicated in various diseases, including cancer, inflammation of tissues and so on. Therefore, it is of great importance to explore a novel method to detect ClO^- and temperature instantly. In this study, we developed a silica nanoparticle-based dual-responsive ratiometric fluorescent sensor (DRFS), whose correlative dual emissions can response to ClO^- and temperature independently and sensitively. The detection limit of DRFS can reach to as low as 26 nM for the detection of ClO^-. And further research demonstrates that DRFS possesses excellent anti-interference feature when other possible interferents exist, and has been successfully applied in ClO^- detection in human serum and recognition of exogenous/endogenous ClO^- in HeLa cells and macrophages by fluorescence microscopic imaging. Moreover, DRFS can also be used as a ratiometric temperature sensor, and the fluorescence intensity ratio (I_{576}/I_{445}) exhibits a linear temperature response in the range from 20 to 60 °C with a change ratio as large as a factor of 5. Based on the above research, the DRFS can be used as versatile fluorescence sensor in various physiological and environmental systems.

1. Introduction

Hypochlorite (ClO^-) is widely existed in the biological and environmental systems [1–3], which plays an important role in our daily life. For example, ClO^- is usually selected as a domestic detergent or a disinfectant for dealing with drinking water, treating wastewater, swimming pool water and others [4–7]. ClO^- is also recognized as natural defense in living organisms due to its prominent antimicrobial properties [8,9] and involved with a wide variety of biological behaviors. However, excessive generation of ClO^- conceals huge health threat to human and animals [10–13]. Experimental investigation has ascertained that superabundant intake ClO^- can result in tissue damage and diseases like lung injury [14], atherosclerosis [15], arthritis [16], neuron degeneration [17] and even cancers [18,19]. On the other hand, temperature, which is one of pivotal physical parameters, plays an important role in chemical and biological systems, such as cellular behaviors and chemical equilibrium [20,21]. The normal metabolism of all cells requires a proper temperature to regulate. However, acatastatic temperature is tightly associated with various pathological cellular dysfunctions [22], which may lead to many diseases such as inflammation of tissues, and cancer [23]. Therefore, it is extremely vital to explore novel strategies to effectively and accurately detect ClO^- and temperature instantly.

Nowadays, a large number of fluorescent sensors for ClO^- or temperature with single emission have been reported [24–31]. Among these traditional sensors [32], they usually suffer from a severe limitation. For example, the change of emission peak

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intensity is easily influenced by various factors including the efficiency of instruments, sensor concentration, external quenchers and complex environmental conditions [33]. These factors would induce a low signal to noise ratio and less efficiency of detection [34,35]. By contrast, ratiometric dual-emission fluorescent sensors can greatly alleviate the above limitations of one emission signal-based measurements due to they do not suffer from deficiencies like stoichiometric imbalance, which will minimize background signals and optimize the detectability of different signals [36–40].

To date, a variety of ratiometric fluorescent sensors for ClO^- or temperature have been already reported [41–48]. Consideration of high correlation of ClO^- and temperature in various ranges of physiological processes and diseases, it is most desirable to develop a multifunctional sensor for real-time detection of them. However, a ratiometric fluorescent sensor with dual-response (ClO^- and temperature) capability in aqueous solutions is still scarce. Therefore, it is highly commendatory to construct ratiometric dual-emission fluorescent sensors with bifunctional sensing properties like ClO^- and temperature.

In this paper, we report on the fabrication of a new silica nanoparticle-based fluorescent sensor, which can be used as a dual-response ratiometric fluorescent sensor (DRFS) for detecting ClO^- and temperature independently and effectively in aqueous solution. The sensor DRFS is consisted of fluorescent silica nanoparticle (FSNP, as hypochlorite sensing unit) and rhodamine B isothiocyanate (RBITC, as temperature sensing unit) (Scheme S1). One of the fluorescent moieties is immune from external environment to be a stable reference signal, while the other one can be activated by external stimuli. As illustrated in Scheme 1, for this sensing platform, the fluorescence quenching phenomenon of the FSNP is observed with addition of strong oxidant ClO^-, while emission intensity from RBITC remains nearly unchanged, resulting in the ratiometric detection of ClO^- in aqueous solution. In addition, the as-prepared DRFS is also used as an excellent ratiometric fluorescent sensor for accurately monitoring of temperature ranging from 20 to 60 °C.

2. Experimental

2.1. Materials

Lipopolysaccharide (LPS) and phorbol myristate acetate (PMA), 3-Aminopropyltrimethoxysilane (APTES), trisodium citrate, rhodamine B isothiocyanate (RBITC), L-cysteine (Cys), glutathione (GSH), Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), hypochlorous acid (HClO), sodium hypochlorite (NaClO), and ferrous sulfate ($FeSO_4$) are purchased from Sigma-Aldrich. Nickelous sulfate ($NiSO_4{\cdot}6H_2O$), calcium chloride anhydrous ($CaCl_2$), sodium chloride (NaCl), cobaltous nitrate ($Co(NO_3)_2{\cdot}6H_2O$), potassium chloride (KCl), Magnesium chloride ($MgCl_2$), Manganese sulfate ($MnSO_4$), Cupric sulfate ($CuSO_4{\cdot}5H_2O$), Zinc chloride ($ZnCl_2$) and Mercury(II) chloride ($HgCl_2$) are of analytical grade reagents. The water used in the whole experiments is dealt with double-distillation and then further purified by a Milli-Q water purification system.

2.2. Preparation of fluorescent silica nanoparticle (FSNP)

In this work, the preparation of FSNP through hydrothermal method was conducted as follows. Firstly, 0.4 g trisodium citrate was dispersed in 10 mL deionized water in a 25 mL two-necked flask, then the transparent solution was stirred for 30 min with the continuous aeration of nitrogen gas of high purity to remove oxygen. Secondly, 2.5 mL APTES was injected into nitrogen-saturated solution and stirred additional 15 min. The mixture solution was transferred into a teflon-lined autoclave of 30 mL and sealed with a stainless steel cover. The reaction kettle was placed in an oven at 160 °C and incubated for 5 h. The reaction solution was cooled to room temperature by natural cooling. Finally, the excessive impurities, such as trisodium citrate and APTES, were removed by dialysis process against water with 500 Da dialysis bags, and the prepared fluorescent FSNP solution was stored at 4 °C for use.

2.3. Synthesis of fluorescent sensor (DRFS) based on FSNP and RBITC

RBITC (5 mg) as fully dissolved in 10 mL as-prepared FSNP solution. The mixture solution was placed in the dark and stirred for 48 h. Afterwards, the unreacted FSNP and RBITC were removed by a dialysis procedure through a porous cellulose membrane (MWCO 500) against the pure water for 24 h. The stable RBITC-functionalized FSNP dispersion, a DRFS for hypochlorite recognition and temperature response, was obtained and kept in the dark for use.

2.4. Spectrum studies

Stock solutions of the sensor DRFS were prepared in deionized water. Test solutions were prepared by using the stock solution and diluting with deionized water. The ClO^- solution of various concentrations was prepared with deionized water. The other solutions (100 μM) were also prepared in deionized water: Cys, GSH, H_2O_2, TBHP, HClO, Fe^{2+}, Ni^{2+}, Ca^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, K^+ and Na^+. For all measurements, the excitation wavelength was 360 nm and for hypochlorite recognition and temperature response.

2.5. Cell viability assay

HeLa cells and macrophages were used to evaluate the cytotoxicity of the sensor DRFS by MTT assay according to ISO 10993-5.

2.6. Cell culture and imaging studies

The living HeLa cells and RAW 264.7 macrophage cells were cultured in culture medium including 10% FBS, 50 unit/mL penicillin, and 50 μg/mL of streptomycin at 37 °C. As to cell imaging studies, cells were seeded in a 80-well plate at a density of 100 cells per well in culture medium and maintained at 37 °C in a 5% CO_2/95% air incubator for 12 h. For evaluating the sensing performance of ClO^-, the living HeLa cells are incubated with DRFS (10 μg/mL) in culture medium for 15 min at 37 °C and then washed by PBS twice. Then, the living HeLa cells were treated with NaClO (20 uM) for another 15 min in culture medium at 37 °C. All of the fluorescence images of living HeLa cells were recorded by a confocal laser scanning fluorescence microscope setup containing an Olympus IX81 inverted microscope with an Olympus FV1000 confocal scanning system.

For RAW 264.7 macrophage cells, the as-prepared cells were incubated with DRFS (10 μg/mL) in culture medium for 15 min at 37 °C and washed by PBS twice. Afterwards, the macrophage cells were incubated with LPS (1 mg/mL) for 5 h at 37 °C, and then further co-incubated with PMA (1.0 mg/mL) and DRFS (10 μg/mL) for 15 min at 37 °C. All the fluorescence images of cells were obtained by a confocal laser scanning fluorescence microscope setup containing an Olympus IX81 inverted microscope with an Olympus FV1000 confocal scanning system after being washed twice.