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The application of natural drug-curcumin in the detection hypochlorous acid of real sample and its bioimaging

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ABSTRACT

The detection property of natural drug-curcumin (**1**) was firstly reported by us. Experimental details showed **1** acts as an excellent UV–vis and fluorescent selectivity, sensitivity probe for the detection of hypochlorous acid, while other reactive oxygen species (ROS) and reactive nitrogen species (RNS) induced no changes in UV–vis spectra and fluorescence quench properties of **1**. A possible detection mechanism is that HOCl oxidized phenol of **1** into quinone resulting in a non-fluorescent curcumin derivative. Furthermore, the ability of probe to detect ClO^- in living cells was also evaluated (HepG2 cells) via a cancelation of the fluorescence. Moreover, the application of **1** in quantitative analysis of hypochlorite in 84 disinfectant was also illustrated.

1. Introduction

Curcumin (**1**) forms the majority of the naturally occurring yellow pigments in the Indian spice turmeric. It has a unique conjugated structure including two methoxylated phenols and the enol form of α-diketone. Because of its particular structure, potential pharmacological effects and negligible cytotoxicity [1], many researches about its property have been reported. Extensive research over the last several decades indicates that **1** possesses potent antioxidant [2–4], anti-inflammatory [5], antitumor [6–8], anti-HIV [9], and antimicrobial [10–12] properties. It has been found that keto-enol-enolate equilibrium of the heptadiene-dione moiety in **1** determines its physiochemical properties. Both phenolic-type natural antioxidants including tocopherol and β-diketone moiety have antioxidative property [3,13]. In our experiments, we found that when **1** encountered with HClO, one part of the o-methoxyphenol in **1** was oxygenated into quinone and then existed as the stable form 4-((1E,4Z,6E)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-3-oxohepta-1,4,6-trien-1-yl)cyclohexa-3,5-diene-1,2-dione (**2**). This is a novel oxidation mechanism reported by us.

Hypochlorite (ClO^-) and its protonated form (HClO) are encountered widely in our daily lives. Sodium hypochlorite is frequently used as the effective components of 84 disinfectant and bleaching agent. In living organisms, hypochlorite is synthesized from H_2O_2 and chloride ions in a chemical reaction catalyzed by the enzyme myeloperoxidase (MPO) [14]. Hypochlorite, because of its strong nucleophilic nonradical oxidability, is a key microbicidal agent that is used for natural defense. Although hypochlorite functions mainly in the prevention of microorganism invasion, increasing evidence suggests that abnormal levels of hypochlorite can lead to tissue damage and diseases such as atherosclerosis, arthritis, and cancers [15–17]. However, the mechanism of action of hypochlorite in these diseases is still not fully understood because of the lack of sensitive and specific probes for detecting. Up to now, the hypochlorite fluorescent probes, which have been synthesized and applied to hypochlorite detection, are most p-methoxyphenol derivatives [18–20], oxime derivatives [21–24] and rhodamine derivatives [25–28] with the predicament of complicated multi-step synthesis, low yield and insurmountable biotoxicity. Herein, we reported the natural drug-curcumin as an excellent selectivity, sensitivity probe for the detection of hypochlorite both in biosystem and industry detection.

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2. Experimental

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma–Aldrich (St. Louis, MO). All chemicals and solvents were of analytical grade without further purification. Deionized water was used to prepare all aqueous solutions. The solutions of anions were prepared from their sodium salts. Superoxide solution ($O_2^{\bullet -}$) was prepared by adding KO_2 (1.0 mg) to dry dimethylsulfoxide (1.0 mL) and stirring vigorously for 10 min. Hydroxyl radical (•OH) was obtained from the Fenton reaction of ferrous perchlorate and hydrogen peroxide. Single oxygen ($^1O_2$) was generated by mixing H_2O_2 with NaOCl sequentially. ROO• was generated from 2,2′-azobis(2-amidinopropane)dihydrochloride. Nitric oxide was generated from SNP (sodium nitroferricyanide (III) dihydrate). SNP in deionizer water was added then stirred for 30 min at 25 °C [19,29,30]. The 84 disinfectant was purchased from manufacturer.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on a Cary 50 Bio UV–visible spectrophotometer. Fluorescence spectra were measured on Cary Eclipse fluorescence spectrophotometer. All fluorescence and UV–vis spectra data were recorded at 10 s after the analytes addition. A PO-120 quartz cuvette (10 mm) was purchased from Shanhai Huamei Experiment Instrument Plants, China. 1H NMR, 13 C NMR spectra were recorded on a Bruker AVANCE-300 MHz and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Melting point (mp) was determined on WRS-2 digital melting point apparatus (Shanghai Physical Optical Instrument Factory). ESI-MS was measured with an LTQ-MS (Thermo) instrument. The ability of probe **1** reacting to hypochlorite in the living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope.

3. Results and discussion

3.1. The selectivity of probe for hypochlorite

Fig. 1a shows the fluorescence changes that **1** undergoes upon the addition of various oxidizing anions and radicals, including ClO^-, H_2O_2, $^1O_2$, NO, $O_2^{\bullet -}$, HO•, $P_2O_7^{4-}$, IO_4^-, $ONOO^-$, ROO•, ClO_2^-, $S_2O_3^{2-}$ in MeOH/HEPES (v/v, 3:1, pH 7.0). The probe **1** displays a fluorescence quench (lex = 420 nm, lem = 544 nm) in the presence of NaClO. However, other anions and radicals induced no changes in the fluorescence emission properties under the same conditions. At the same time, the competition of other anions and radicals on the determination of ClO^- was examined. Fig. 1b displayed the fluorescence responses of the probe–ClO^- to the presence of various anions and radicals. The emission quench of probe in response to ClO^- was primarily unaffected in the presence of various competitive species.

3.2. pH and time dependent of determination

The optimum pH for the system was investigated using solution with pH values between 2.0 and 13.0. Fig. 2 showed the fluorescence emission spectra obtained for the free probe and its product induced by hypochlorite in different pH values. The intensity of emission at 544 nm decreased in strong alkaline solutions (pH 12–13) compared to that at other pH values. Hypochlorite partially quenched the fluorescence of probe in the range of pH 2–6. As we know, HClO is a stronger oxidant in strongly acidic solutions than

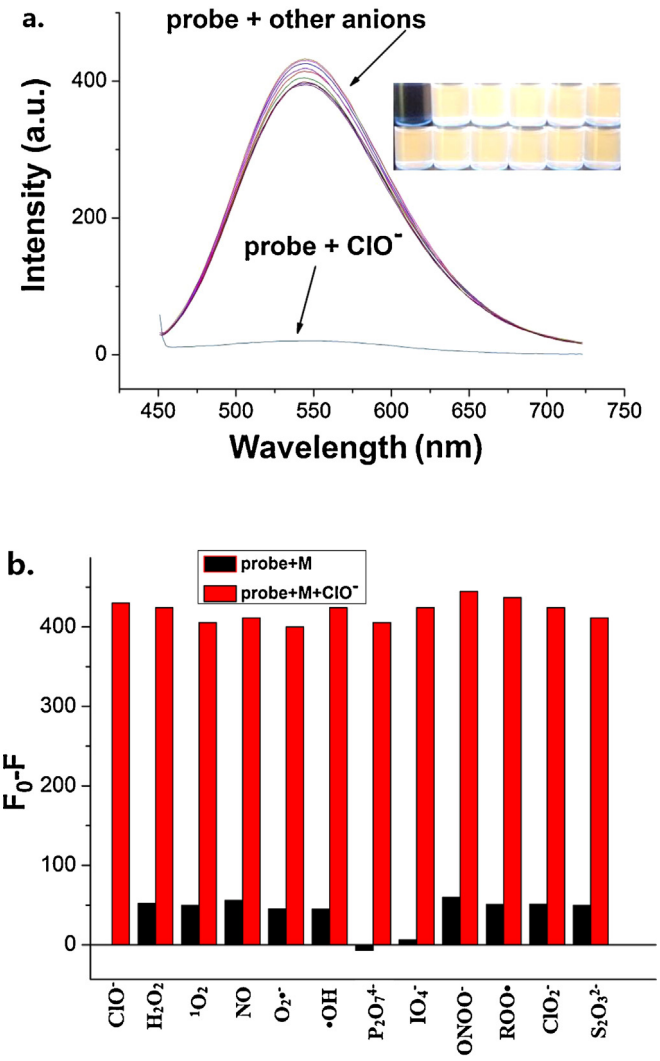

Fig. 1. (a) Fluorescence spectra of **1** (5 μM) with ClO^- (5 μM) various analytes (100 μM) in MeOH/HEPES (v/v, 3:1, pH 7.0) (λ_{ex} = 420 nm, slit: 5 nm/5 nm), inset: photograph showing the fluorescence change for ClO^- (colorless) and the other analytes (green) under illumination with a 365 nm UV lamp. (b) Relative fluorescent intensity (λ_{ex} = 420 nm, λ_{em} = 544 nm) of the system (black bar: probe + various anions and radicals, red bar: probe + various anions and radicals + ClO^-). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in weakly acidic solutions. However, in strongly acidic solutions, HClO will decompose rapidly. Therefore neutral and alkalescence solutions were suitable for the detection of HClO.

Time-dependent modulations in the fluorescence spectra of **1** were monitored in the presence of 10 equiv. of NaClO. The kinetic study showed that the reaction was complete within 6 s, indicating that probe **1** reacts rapidly with ClO^- under the experimental conditions (Fig. S1).

3.3. UV–vis and fluorescence spectra of detecting hypochlorite

As a typical oxidizing agent, ClO^- was used to further examine the UV–vis response of probe **1**. Fig. S2 shows the change in the UV–vis spectra when ClO^- was added to MeOH/HEPES (v/v, 3:1, pH 7.0) solution containing **1** (10 μM). With increasing ClO^- concentration (0–1 equiv.), the absorption peak at 430 nm gradually decreased and the absorption peak enhanced at 262 nm. A well-defined isosbestic point was noted at 320 nm, which may indicate the formation of single new species. When the