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A novel recognition mechanism supported by experiment and theoretical calculation for hypochlorites recognition and its practical application

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ABSTRACT

We have developed two new colorimetric and fluorescent probes for hypochlorite based on a novel recognition mechanism: amido oxidized nitroso-group by hypochlorites, which realized the preparation of nitroso compounds. Furthermore, ^{1}H NMR, ESI-MS and theoretical calculation proved the recognition mechanism. In addition, the probe **2** was applied in practical applications such as detecting the hypochlorite concentration of sodium hypochlorite disinfectant and bioimagings.

1. Introduction

The detection and quantification of inorganic anions have been invigorated over the past years from environmental, biological, and medical aspects. And a variety of probes that are able to selectively recognize anions were designed [1–8]. As one of the reactive oxygen species (ROS), hypochlorite is generated from the reaction of H_2O_2 and chloride ion catalyzed by myeloperoxidase (MPO) enzyme in organisms [9,10]. In the physiological pH solution, HOCl is partially dissociated into OCl^-, and it plays a crucial role in vivo due to its antibacterial properties [11,12]. However, the abnormal production of hypochlorite can lead to tissue damage and diseases, such as arthritis [13], neuron degeneration [14], cardiovascular diseases [15], and cancer [16,17]. On the other hand, hypochlorite is also widely used in our daily life. Sodium hypochlorite is the most commonly used chlorinated substance and extensively used as a household cleaning agent and as disinfectant for treatments including drinking water, swimming pool water, treated wastewater for non-potable reuse and others [18–22]. As we know, an excess level of ClO^- is harmful to the health of human beings. Thus, it is essential to design chemosensors for the detection of $HOCl/OCl^-$. Some successful examples have been reported recently. The most reported oxidizable moieties by different oxidants bearing oxidizable auxochromic groups, such as thiol [23,24], phenol, hydroxamic acid [25], dibenzoylhydrazine [26], oxime derivatives [27,28], oxazine-conjugated nanoparticle [9], and different metal complexes [29,30].

Rhodamine-based dyes have been investigated extensively as fluorescence reporting groups owing to their excellent spectroscopic properties such as long absorption and emission wavelength, large absorption coefficient and high fluorescence quantum yields.

Herein, we developed two new colorimetric probes for hypochlorite based on *p*-nitroaniline or rhodamine 110 being oxidized into nitroso compounds by sodium hypochlorite. It is well-known that nitroso compounds, extremely versatile reagents, are extensively used in organic synthesis [31,32]. Classic methods for the preparation of aromatic nitroso compounds are oxidation of anilines with peracids [33], particularly caro acid [34], perbenzoic acid [35,36], trifluoroperacetic acid [37] or more recently molybdic peroxo-complexes [38]. And in this work, we developed two commercially available aniline compounds **1** and **2** (Scheme 1) as hypochlorite probes, which were oxidized into nitroso compounds. Besides, it also provided a simple and new method for the synthesis

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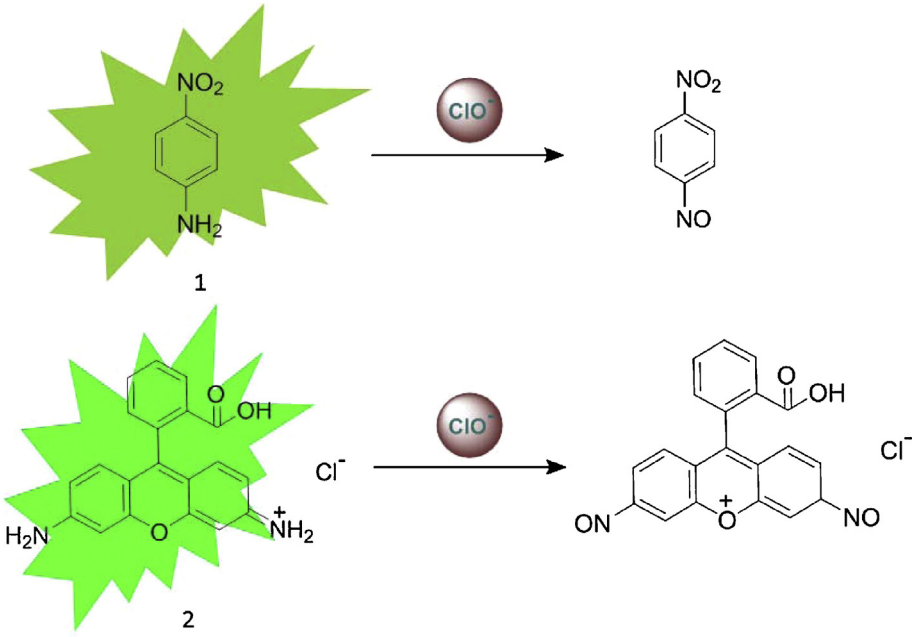

Scheme 1. The synthesis of nitroso compounds by hypochlorite under mild conditions.

of nitroso compounds by hypochlorite under mild conditions (i.e., at ambient temperature) in organic synthesis.

2. Materials and methods

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma–Aldrich (St. Louis, MO). *p*-Nitroaniline and rhodamine 110 were purchased from Aladdin Industrial Corporation (Shanghai, China). Anionic salts were purchased from Shanghai Experiment Reagent Co., Ltd., China. All other chemicals used were of analytical grade. Deionized water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in HEPES (10 mM, pH 7.4) buffer. HEPES buffer solutions were obtained by adding 1 M NaOH solution into 10 mM aqueous HEPES using a Mettler Toledo pH meter.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. The UV–visible spectra were recorded on a Cary 50 Bio UV–Visible spectrophotometer. Fluorescence spectra were measured on Cary Eclipse fluorescence spectrophotometer. The reported kinetic data were determined at 25 °C. The ability of probe 2 to detect ClO^- within living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope. All data were treated with the Origin 8.0 program. ^1H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz and 75 MHz NMR spectrometer, respectively. Chemical shifts are given in parts per million downfield from tetramethylsilane (0.0 ppm) for spectra. ESI-MS was measured with an LTQ-MS (Thermo) instrument.

2.3. Synthesis of the nitroso compounds by hypochlorite

The probe **1** was dissolved in methanol (10 mL), excessive sodium hypochlorite was poured into the solution. The mixture was stirred at ambient temperature for 5 min to complete the reaction. The final mixture was diluted by water (10 mL) and extracted with trichloromethane (3× 15 mL). The organic layer was concentrated under reduced pressure evaporation to give the desired the probe **1**+ ClO^- product. Similarly, the probe **2** was dissolved in methanol (10 mL), excessive sodium hypochlorite was poured into the solution. The mixture was stirred at ambient temperature for 5 min to complete the reaction. The final mixture was diluted by water (10 mL) and extracted with trichloromethane (3× 15 mL). The organic layer was concentrated under reduced pressure evaporation to give the desired the probe **2**+ ClO^- product (Scheme 1).

2.4. Measurement procedure

The UV–vis procedures were shown as follows: into a 10 mM, pH 7.4 HEPES buffer solution containing 80.0 μM probe **1** (or 7.0 μM probe 2), ClO^- sample was gradually titrated, respectively. All UV–vis spectra data were recorded at 5 min after ClO^- addition.

The fluorescence procedures were as follows: into a 10 mM, pH 7.4 HEPES buffer solution containing 50.0 μM probe **1** (or 0.4 μM probe **2**), ClO^- sample was gradually titrated, respectively. All fluorescence spectra data were recorded at 5 min after ClO^- addition.

The HepG2 cells were grown in 1× SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferric sodium salt) at 30 °C. The HepG2 were treated with 1.0 μM of probe **2** in culture media for 30 min at 37 °C and washed three times with phosphate-buffered saline (PBS). For the control experiment, the cells were treated with 40 μM N-ethylmaleimide (NEM) in culture media for 30 min at 37 °C. After washing with PBS to remove the remaining NEM, the cells were further incubated with 1.0 μM of probe **2** in culture media for 30 min at 37 °C.

Theoretical predictions based on DFT and TDDFT were explored at the m062x/6-31+G (d) level.

3. Results and discussion

3.1. Competitive binding assay and selectivity experiment for ClO^-

Selectivity is a major issue in the field of anion sensing. Various analytes such as F^-, CN^-, $P_2O_7^{4-}$, ClO_3^-, ClO_2^-, H_2O_2, HSO_3^-, MnO_4^-, NO_2^-, SCN^-, ClO_4^-, AcO^-, S^{2-}, N_3^-, $ROO^\bullet$, $NO^\bullet$ sensing