Sensors and Actuators B 265 (2018) 365–370

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Novel diaminomaleonitrile-based fluorescent probe for ratiometric detection and bioimaging of hypochlorite

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ARTICLE INFO

Article history:
Received 1 December 2017
Received in revised form 21 January 2018
Accepted 25 January 2018
Available online 3 February 2018

Keywords:
Hypochlorous acid
Ratiometric Fluorescence
Colorimetric fluorescent
Cell imaging

ABSTRACT

Highly sensitive and selective detection for hypochlorite anion (OCl^-) is of extreme significance due to its important role and function in biological systems. Herein, a diaminomaleonitrile derivative (DMD) has been developed as a novel ratiometric and colorimetric fluorescent probe for OCl^- detection. Consistent with the UV–vis spectral change, the probe DMD exhibited promptly a color change from canary yellow to gules upon the addition of OCl^-, which could be easily observed by naked-eyes. In addition, the detection limit was found to be 0.5 nM, which is much lower than that of many reported OCl^- probes. More importantly, owing to its excellent sensing properties and negligible cytotoxicity, probe DMD can be conveniently applied to image intracellular OCl^- in living cells, indicating it a promising tool in biological applications.

1. Introduction

Though widely encountered as bleaching and cleaning agent in our daily life, hypochlorite anion (OCl^-) has recently attracted attention for its critical role in biological systems [1]. For instance, endogenous hypochlorite produced from the reaction of chloride ion and hydrogenperoxide catalyzed by the enzyme myeloperoxidase (MPO) which contributes to destroying the invading bacteria and pathogens powerfully [2–4]. However, abnormal OCl^- level could result in many diseases such as cancer [5], Alzheimer's disease [6], neuron degeneration [7], osteoarthritis [8], and renal disease [9,10]. Therefore, there is growing interest in the development of artificial probe for OCl^- monitoring with high sensitivity, particularly in living cells. So far, many methods have been reported for the determination of OCl^- level [11–19]. Fluorescent analysis method has been regarded as one of the most effective technique for OCl^- detection involving outstanding advantages such as low cost, operation simplicity, real-time imaging and high sensitivity [20,21].

Various fluorescent probes have been designed and employed for OCl^- detection both in vitro and in vivo imaging [22–32] and constructed a series of intensity-based fluorescent probes. Nonetheless, some of them might be easily affected by the variations in environment, concentration and excitation intensity that limited their practical applications [33,34]. Ratiometric fluorescent probe are favored for its advantage on recorded ratio signal of two emission intensities at different wavelength affording a built-in correction, which can solve the problems exist in intensity-based probes very well [35–40]. At present, much progress has been contributed significantly to the exploitation of ratiometric probes for OCl^- detection, unfortunately, most of them require complicated synthesis and rigorous conditions. Therefore, adequate studies on the development of advanced fluorescent probe for simply OCl^- detection is highly demanded.

In this work, a novel diaminomaleonitrile derivative (DMD) was introduced by one-step reaction (Scheme 1) to achieve radiometric fluorescent signal for the detection of hypochlorite. Upon the addition of OCl^-, the fluorescence emission peak of DMD at 521 nm was gradually weakened with a new peak located at 635 nm appeared and gradually enhanced correspond to the solution of DMD changed from canary yellow to gules which provided a new ratiometric and colorimetric detection approach for hypochlorite.

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https://doi.org/10.1016/j.snb.2018.01.202
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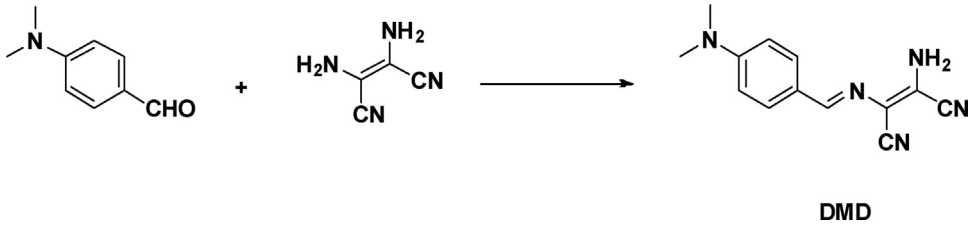

DMD

Scheme 1. Synthesis route of DMD probe.

2. Experimental

2.1. Apparatus and chemicals

^{1}H NMR and ^{13}C NMR spectra of samples were recorded on a Bruker AN-400 MHz (Bruker, AVANCE III HD) instrument. Ultraviolet-Visible (UV–vis) absorption spectra were measured with a Varian Cary 50 spectrophotometer (USA, Agilent) at 1 cm of the light path length. Fluorescence spectra were recorded on Varian cary eclipse fluorescence spectrophotometer (USA, Agilent) with an excitation wavelength of 385 nm. Solid state fluorescence data were recorded on HORIBA Scientific FluoroMax-4 with an excitation wavelength of 380 nm. Mass spectroscopy data of the products were collected on direct analysis in real time (DART) ion source (Ion sense Inc., USA) coupled to a benchtop Orbitrap Fusion Lumos (Thermo Fisher Scientific, USA) mass spectrometer, which incorporates a segmented quadrupole mass filter with improved selectivity and ion transmission. All fluorescence imaging experiments were conducted under a A1 confocal laser scanning microscope (Nikon, Japan).

4-(Dimethylamino)benzaldehyde, 2,3-diaminomaleonitrile, hydrogen peroxide, sodium hypochlorite, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromi de (MTT) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China) and used without further purification. Ethanol and DMSO were analytically pure reagents and distilled before used. Various testing species including H_2O_2, OCl^-, $TBuO^-$, HO^-, $^1O_2$, NO, $ONOO^-$ and NO_2^- were prepared in solutions according to the following methods, respectively: T-BuO^- from Aladdin Co. Ltd was diluted to the required concentration; HO^- was generated from Fenton reaction between Ferrous solution; $^1O_2$ was produced via the reaction of H_2O_2 with NaOCl; NO is emitted from 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5); $ONOO^-$ source was replaced by the 3-morpholinosydnonimine hydrochloride (SIN-1, 50.0 μM); The source of NO_2^- was prepared from $NaNO_2$. All other ion salts were purchased from Nanjing Chemical Reagent Co., Ltd. (China). Double-distilled water was used throughout all the experimental solutions. All samples were prepared at room temperature and were shaken for 1 min before the test.

2.2. Synthesis of DMD

0.15 g 4-(dimethylamino)benzaldehyde (1 mmol) was dissolved in 20 mL ethanol solution, then 0.12 g diaminomaleonitrile (1.1 mmol) was added into the solution and the mixture was heated at reflux and stirred for 12 h. After reaction system was cooled to room temperature and yielded a yellow precipitate which was collected by filtration, washed with cold ethanol, and dried in vacuo to afford probe DMD as a yellow powder in 85% yield (0.19 g). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.11 (s, 1H), 7.84 (d, 2H. J = 8.0 Hz), 7.47 (s, H), 6.76 (d, 2H, J = 8.0 Hz), 3.03 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 55.8, 152.9, 131.3,124.6, 123.5, 115.5, 114.5, 111.9, 104.6. HRMS (ESI) calcd. for $[M]^+$ 238.1075, found 238.1071.

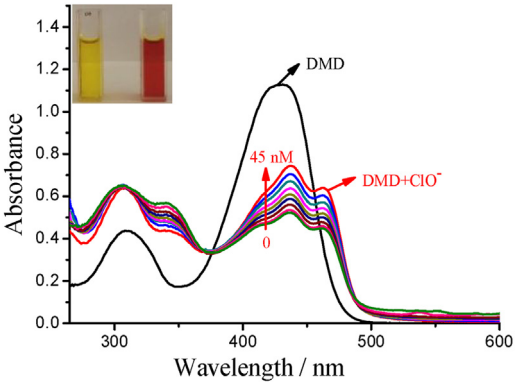

Fig. 1. UV–vis spectra of DMD (10 mM) (black line) and DMD (10 mM) with hypochlorite (0–45 nM) in DMSO–water solution (1: 1, v/v, 50 mM PBS buffer solution at pH 7.4). Inset: images of DMD (canary yellow, left) and in the absence OCl^- under day light (gules, right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. UV–vis and fluorescence spectra of DMD with OCl^-

Double-distilled water and analytical purity DMSO were used to prepare solvents for the absorption spectra studies. Compound DMD was dissolved in DMSO/PBS buffer solution (5:5, V/V; pH 7.4) to prepare the concentration of 10 μM. The other analysts were dissolved in HEPES buffer (10 mM, pH level of 7.4, 2% DMSO) to prepare the stock solutions. The resulting solutions were shaken well and incubated for 1 h at room temperature before recording spectra data.

2.4. Cytotoxicity studies

The methyl thiazolyl tetrazolium (MTT) assay was used to measure the cytotox- icity of DMD in A549 cells. A549 cells were seeded in a 96-well plate (90 μL $well^{-1}$) overnight and DMD suspensions with different concentrations (20, 40, 60, 80, and 100 μg mL^{-1}) were then added. The cells were cultivated for 24 h, and 20 μL of 1 mg/mL MTT solution was then added to each cell well. After the cells were incubated for 4 h, the culture medium was discarded, and 150 μL DMSO solution was added. The resulting mixture was shaken for 15 min in dark at room temperature, and its optical density (OD) was measured on a microplate reader (USA, Thermo TY10FC).

2.5. Cellular imaging

Human A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin in humidified environment of 5% CO_2. The DMD suspension was injected into the well of a chamber slide with the final DMD concentration of 20 μg mL^{-1}. After incubation for 24 h, the cells were washed 3 times using phosphate buffered saline. The fluorescence images were acquired using an excitation wavelength of 488 and 561 nm.