Sensors and Actuators B 240 (2017) 625–630

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

# Lighting up carbon monoxide in living cells by a readily available and highly sensitive colorimetric and fluorescent probe

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ARTICLE INFO

*Article history:*
Received 7 July 2016
Received in revised form 30 August 2016
Accepted 6 September 2016

*Keywords:*
Carbon monoxide
Fluorescent probe
Tsuji–Trost reaction
Living cells

ABSTRACT

Carbon monoxide (CO) is an important gasotransmitter in living systems. The biological detection of this gasotransmitter is still in its infancy and urgently needs probes. In this paper, a novel fluorescent probe system, which is based on the $Pd^0$-mediated Tsuji–Trost reaction, is reported for rapid detection of CO both in *in vitro* and in living cells. This probe system is readily available and shows excellent sensing properties for CO including rapid and distinct colorimetric and fluorescent turn-on responses, high selectivity and sensitivity with a low detection limit (25 nM) in aqueous solution under mild conditions. In addition, fluorescent imaging of CO in living cells can be conveniently achieved by a low dose of this probe system.


## 1. Introduction

Carbon monoxide (CO) is a well-known toxic gas. Owing to its colorless and odorless nature, CO is often a silent and invisible threat that could lead to fatal consequences on inhalation [1]. However, this cannot change the fact that CO can be produced during the haem catabolism in the human body and other biological systems [2]. More importantly, studies have revealed that CO is a very important biological signalling molecule (gasotransmitter) that implicated in vasodilatation, neurotransmission, anti-inflammatory and antiapoptotic processes [3]. In addition, CO has also been recognized as a therapeutic molecule that involved in a multitude of defense mechanisms under physiological and pathological conditions [4]. Due to these benign roles, CO has captivated considerable attention during the past few years, and there is a strong demand for the further understanding of its functions in biological systems [5–7]. However, lack of efficient ways for selective monitoring of this transient gas molecule in living systems has been one major obstacle in this research area [8]. Although some detection methods for CO have been reported, such as gas chromatography [9], chromogenic detection [10–13], electrochemical assays [14], these methods are usually unsuitable for real-time detection of CO in living cells [15]. Therefore, it is important and urgent to develop convenient, sensitive and selective detection method for rapid detection of CO both in *in vitro* and in living systems, either for security reasons, or for biological research.

Recently, fluorescence detection based on fluorescent probes has emerged as powerful tools to monitor and detect various biologically important molecules due to its convenient, sensitive and noninvasive manner [16]. However, fluorescent detection of CO in living systems is still in its infancy to date [17–19]. Although several fluorescent detection systems for CO have been developed in the past ten years [20–27], it is only in the last few years that the fluorescent detection of CO in living systems has been addressed. In 2012, He et al. [28] firstly described a genetically encoded fluorescent protein that was able to selectively image CO in living cells. Shortly after this elegant work, Chang and co-workers [29] reported a palladium-BODIPY complex for imaging CO in living cells. After that, several more probes have been developed and applied for cell-imaging of CO [30–32]. However, most of these probes still display certain drawbacks such as difficult to synthesize, poorly resolved changes in color or emission, modest selectivity, long response times or using UV light (<400 nm) for excitation, as has been highlighted very recently [19]. Obviously, it is still urgent to develop new fluorescent systems for CO detection with improved sensing properties.

Herein, we report a new fluorescent probe system for detection of CO. This probe system is composed of two molecules, an allyl chloroformate functionalized 3-benzothiazolyl-7-hydroxycoumarin (BTHC) as the CO signalling molecule (BTHC-CO) and $PdCl_2$ as an additive (Scheme 1). Our studies showed this probe system (BTHC-CO + $PdCl_2$) have several appealing merits: (1) It is readily available and highly selective and sensitive for CO; (2) It shows a rapid, distinct colorimetric and fluorescent turn-on response for CO; (3) It has a low detection limit (25 nM) for CO; (4) It can be used in a low dosage (1 μM level based on BTHC-CO)

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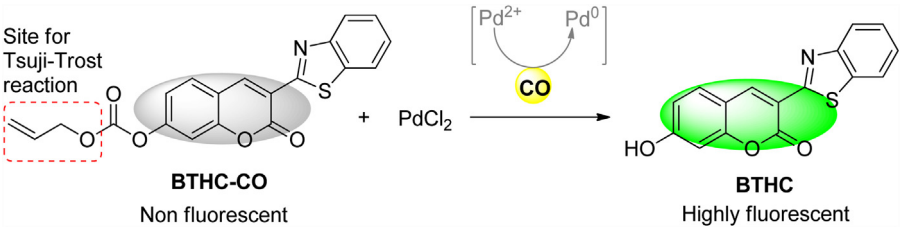
http://dx.doi.org/10.1016/j.snb.2016.09.023
0925-4005/




**Scheme 1.** Detection of CO by a mixture of BTHC-CO and $PdCl_2$ using Tsuji–Trost reaction.

for fluorescent imaging of micromolar concentration levels of CO in living cells. Based on these excellent sensing properties, this fluorescent probe system provided an attractive new detection method for CO.

## 2. Material and methods

### 2.1. Materials and instrumentation

All chemicals and solvents were purchased from commercial suppliers and used without further purification. BTHC was prepared according to our published procedure [33]. $^1$H NMR and $^{13}$C NMR spectra were recorded on a on a Bruker AMX-500 NMR spectrometer, and resonances ($\delta$) are given in parts per million relative to tetramethylsilane (TMS). Coupling constants ($J$ values) are reported in hertz. The low-resolution MS spectra were performed on an electron ionization mass spectrometer. High-resolution mass spectrometry (HR-MS, ESI) spectra were obtained on a Bruker microTOF-Q instrument. UV–vis and fluorescence spectra were recorded on an Agilent Cary-100 UV–vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. Cell imaging was performed in an inverted fluorescence microscopy with a 20× objective lens.

### 2.2. Synthesis of BTHC-CO

BTHC-CO was prepared according to the published procedure [34]. To a solution of BTHC (147 mg, 0.5 mmol) in dry dichloromethane (5 mL) was added allyl chloroformate (180 mg, 1.5 mmol) and $Et_3N$ (150 μL). The resulting mixture was stirred at room temperature until the reaction was complete. Water (10 mL × 3) was used to wash the resulting solution three times, and the dichloromethane phase was dried over $Na_2SO_4$. After filtered and removal of the organic solvent, a yellow-green solid product was formed, which can be further purified by recrystallization from ethanol to afford the pure product (150 mg, 86%). Mp: 99.5–100.6 °C. TLC (silica plate): $R_f$ ~0.77 (petroleum ether: ethyl acetate 3:1, v/v). $^1$H NMR (500 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.09 (d, $J$ = 8.1 Hz, 1H), 7.98 (d, $J$ = 7.8 Hz, 1H), 7.74 (d, $J$ = 8.5 Hz, 1H), 7.54 (t, $J$ = 7.5 Hz, 1H), 7.43 (t, $J$ = 7.4 Hz, 1H), 7.35 (s, 1H), 7.27 (d, $J$ = 8.4 Hz, 1H), 6.00–6.08 (m, 1H), 5.49 (d, $J$ = 17.2 Hz, 1H), 5.40 (d, $J$ = 10.3 Hz, 1H), 4.81 (d, $J$ = 5.6 Hz, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.6, 159.5, 154.4, 154.3, 152.43, 152.4, 140.7, 136.8, 130.6, 130.3, 126.6, 125.5, 122.9, 121.8, 120.2, 119.8, 118.6, 118.9, 109.7, 69.8. EI-MS: $m/z$ found 379.20 ($M^+$). HR-MS (ESI): calcd for $C_{20}H_{14}NO_5S^+$ ($M+H^+$): 380.0587; found 380.0592 and calcd for $C_{20}H_{13}NO_5SNa^+$ ($M+Na^+$): 402.0407; found 402.0412.

### 2.3. Optical studies

Stock solutions of BTHC-CO (1 mM), $PdCl_2$ (2 mM) and CORM-3 (5 mM, CORM-3 is a commercially available carbon monoxide-releasing molecule with chemical name of tricarbonylchloro(glycinato)ruthenium(II), CAS: 475473-26-8) were prepared respectively in HPLC grade DMSO and used freshly. Stock solutions (5–10 mM) of the analytes including NaHS, Cys, Hcy, GSH, Gly, Glu, Trp, Ile, Lys, NaF, NaCl, NaBr, NaI, $Na_2SO_4$, $Na_2HPO_4$, $NaHCO_3$ were prepared in ultrapure water. ROS/RNS species such as $ClO^-$, $H_2O_2$, $NO_2^-$, NO, $ROO^\bullet$, $^tBuOO^\bullet$ and $^\bullet OH$ were prepared according our published procedure and used freshly [35,36]. For a typical optical study, a solution containing BTHC-CO (5 μM) and $PdCl_2$ (10 μM) in DMSO-PBS buffer solution (1:9, v/v, 10 mM PBS, pH 7.4) was prepared. Then 3.0 mL of the solution was placed in a quartz cuvette. The UV–vis or fluorescent spectra were recorded upon addition of an analyte of interest at 37 °C (controlled by a temperature controller).

### 2.4. Imaging of CO in living cells

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% $CO_2$, water saturated incubator at 37 °C, and then were seeded in a 12-well culture plate for one night before cell imaging experiments. In the experiment of cell imaging, as a control, living cells were incubated with a mixture of BTHC-CO (1 μM) and $PdCl_2$ (2 μM) at 37 °C for 30 min and washed with PBS for three times, and then imaged immediately. For imaging of CO, HeLa cells were pre-treated with a mixture of BTHC-CO (1 μM) and $PdCl_2$ (2 μM) for 30 min at 37 °C, and then were incubated with CORM-3 (10–30 μM) for 30 min at 37 °C. The imaging of CO was then carried out after washing the cells with PBS buffer.

## 3. Results and discussion

### 3.1. Probe design and synthesis

Recent work have shown that the well-known $Pd^0$-mediated Tsuji–Trost reaction [37] can be used as an attractive method to sense $Pd^0$ in various fluorescent systems [38–41]. These systems involve the installation of allyl groups onto phenolic fluorophores, and utilize the $Pd^0$-mediated Tsuji–Trost reaction to remove these allyl groups and release the fluorophores, thus producing fluorescence signal changes. Since $Pd^{2+}$ can be readily reduced by CO to generate $Pd^0$ in-situ, this principle has also been successfully used to develop fluorescent probes for CO in very recently [31,32]. On the other hand, coumarin dyes are widely used to develop fluorescent probes [42–45]. Our interest in the development of highly selective fluorescent probes by using the highly bright and fluorescent coumarin dye BTHC [33,46] ($\Phi_F$ = 0.56) prompted us to investigate its possibility to create highly sensitive fluorescent probes for CO. After searching the literature, we found that BTHC-CO has been reported to be a highly selective and sensitive fluorescent turn-on probe for $Pd^0$ over $Pd^{2+}$ [34], however, the sensing ability of BTHC-CO for CO is completely unknown. We thought that BTHC-CO might be used for sensing CO. Thus, BTHC-CO was chosen in this work.

BTHC-CO was then prepared from the readily available BTHC and allyl chloroformate in dry dichloromethane under basic