Dyes and Pigments 148 (2018) 286–291

Contents lists available at ScienceDirect

Dyes and Pigments

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

Colorimetric and fluorescent probe for real-time detection of palladium (II) ion in aqueous medium and live cell imaging

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ARTICLE INFO

Article history:
Received 12 July 2017
Received in revised form
28 August 2017
Accepted 29 August 2017
Available online 14 September 2017

Keywords:
Coumarin oxime
Sulfur atom
Palladium (II) ion
Aqueous solution
Fluorescence imaging

ABSTRACT

A novel fluorescent and colorimetric sensor (**WM-S**) was synthesized from coumarin oxime and 2-chloroethyl methyl sulfide in a straightforward manner. The sensor displayed high selectivity for detection of Pd^{2+} over other metal ions (Cu^{2+} and Hg^{2+} in particular). The probe also exhibited high sensitivity for recognition of Pd^{2+} in aqueous media and can detect trace amounts of Pd^{2+} in real time, with a detection limit as low as 41.5 nM. Fluorescence imaging experiments of Pd^{2+} in HeLa cells indicated that the sensor can penetrate cell membranes and effectively detect intracellular Pd^{2+}.

1. Introduction

With rapid industrial development, pollution due to heavy metal ions has created widespread concern. Palladium, one of the platinum-group elements, has been widely used in various industries like jewelry, medical, electronics, electrical, catalytic converters and the fuel cell industry [1,2] and is one of the most important catalysts in the vehicle exhaust catalyst systems [3]. During the normal course of the vehicle, the discharge rate of palladium is 0.1–0.8 μg km^{-1} car^{-1} [4]. However, a high level of residual palladium is discharged into the environment, which will be eventually consumed by humans via inhalation and ingestion. A recent study has shown that palladium nanoparticles could penetrate the skin and have a potential long-term effect [5]. Palladium strongly binds thiol-containing biomolecules which could disrupt human physiological processes [6–8]. Moreover, palladium ions possess well-documented sensitization potential [9–11]. Therefore, efficient methods for detection of palladium species in environmental and biological samples need to be developed.

There are many established, traditional methods for the detection of palladium species, such as atomic absorption spectrometry [12], time-of-flight resonance ionization mass spectrometry [13] and X-ray fluorescence [14]. However, these methods are time-consuming and require expensive instruments as well as highly skilled operators. In recent years, fluorescence-based techniques have emerged as powerful alternatives to monitor and sense palladium species because of their low cost, high selectivity, excellent sensitivity, low detection limits, operational simplicity, and biological applications [4,15,16]. One popular strategy is to take advantage of palladium-catalyzed reactions to design reactive palladium sensors [17–29]. This method is highly selective and irreversible, and has low detection limits and longer response times; thus, this method is not conducive to real-time detection of palladium. Furthermore, palladium can coordinate with the ligands containing N, O, S, or P and other solitary electronic structures, which may result in a change in color or fluorescence [30–39]. The coordination sensors respond quickly and are susceptible to external influences. Thus, design and synthesis of coordination sensors for real-time detection of palladium species with high selectivity, excellent sensitivity, and in-situ imaging in cells and tissues is necessary. In this paper, we take coumarin as a fluorophore, connecting sulfur atom to coumarin oxime via flexible chain for the design and synthesis of a novel Pd^{2+} fluorescent sensor (**WM-S**). The experimental results confirmed the high selectivity of the sensor **WM-S** towards Pd^{2+} as a result of the color change and fluorescence quenching in the presence of other

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http://dx.doi.org/10.1016/j.dyepig.2017.08.063
0143-7208/

common interfering metal ions, in particular, Cu^{2+} and Hg^{2+}. Furthermore, the detection of Pd^{2+} is rapid and sensitive in aqueous media, with a detection limit as low as 41.5 nM. Moreover, the sensor **WM-S** can penetrate cell membranes and can be applied in intracellular Pd^{2+} detection and imaging in living cells.

2. Experimental section

2.1. Materials and equipment

All reagents and solvents were purchased from commercial sources and used as received without further purification. UV–Vis absorption spectra were recorded using a PGENERAL T6 spectrophotometer. Fluorescence spectra were recorded using a Hitachi F-4500 fluorescence spectrophotometer. ^1H and ^{13}C NMR spectra were recorded using a Bruker Avance AVII-300 MHz spectrometer. High-resolution mass spectra (HRMS) were recorded on an Agilent ESI-Q-TOF mass spectrometer. Live cell imaging was performed with Olympus IX73.

2.2. Synthesis

2.2.1. Synthesis of (E)-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde oxime (**2**)

Compound **2** was synthesized according to reported procedure [40]. Compound **1** (2.5 g, 10 mmol) was dissolved in EtOH (200 mL), then K_2CO_3 (4.3 g, 30 mmol), hydroxylamine hydrochloride (2.2 g, 30 mmol), and piperidine (1 mL) were added. The mixture was stirred vigorously at room temperature for 2 h. After completion of the reaction, solvent was removed by vacuum rotary evaporation. Then DCM (300 mL) was added, and washed three times with aq. sat. brine solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and was evaporated to obtain a crude product, which was then recrystallized in ethanol to yield 2.4 g yellow solid at 92% yield. m.p. 199–200 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 8.27 (s, 1H, 4-H), 7.99 (s, 1H, -CH=N-), 7.49 (s, 1H, N-OH), 7.31 (d, J = 8.8 Hz, 1H, 5-H), 6.59 (dd, J = 8.9, 2.4 Hz, 1H, 6-H), 6.49 (d, J = 2.2 Hz, 1H, 8H), 3.43 (q, J = 7.1 Hz, 4H, -N($\underline{CH_2}CH_3)_2$), 1.23 (t, J = 7.1 Hz, 6H, -N($CH_2\underline{CH_3})_2$).

2.2.2. Synthesis of (E)-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde O-(2-(methylthio)ethyl) oxime (***WM-S***)

Compound **2** (0.26 g, 1 mmol), 2-chloroethyl methyl sulfide (0.22 g, 2 mmol), K_2CO_3 (0.42 g, 3 mmol) were placed in a 100-mL Schlenk tube under argon atmosphere, then anhydrous DMF (25 mL) was added and stirred for 12 h at 100 °C. After cooling the reaction mixture to room temperature, it was poured into ice water (200 mL) and the resulting precipitate was filtered. The precipitate was washed several times with water and dried. The crude residue was purified by flash chromatography affording 0.15 g yellow solid in 45% yield. m.p. 101–103 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H, 4-H), 8.08 (s, 1H, -CH=N-), 7.33 (d, J = 8.9 Hz, 1H, 5-H), 6.61 (dd, J = 8.9, 2.5 Hz, 1H, 6-H), 6.49 (d, J = 2.5 Hz, 1H, 8-H), 4.33 (t, J = 6.9 Hz, 2H, -O-$\underline{CH_2}$-CH_2-S-), 3.44 (q, J = 7.1 Hz, 4H, -N($\underline{CH_2}CH_3)_2$), 2.84 (t, J = 6.9 Hz, 2H, -O-CH_2-$\underline{CH_2}$-S-), 2.20 (s, 3H), 1.24 (t, J = 7.1 Hz, 6H, -N($CH_2\underline{CH_3})_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.04, 155.92, 150.37, 143.25, 137.77, 128.91, 110.78, 108.41, 107.45, 96.20, 72.37, 43.93, 31.93, 15.02, 11.46; HRMS calcd for $C_{17}H_{23}N_2O_3S$ $[M+H]^+$: 335.1424, found 335.1422; IR (KBr, cm^{-1}): 2970, 2923, 1715, 1604, 1578, 1518, 1445, 1414, 1351, 1300, 1253, 1193, 1134, 1080, 1013, 966, 872, 839, 797, 768, 662, 632, 510, 470.

2.3. Preparation of ***WM-S*** and various ions stock solutions

WM-S stock solution was prepared at the concentration of 1.0×10^{-3} M in EtOH and then diluted to a desired concentration.

Unless otherwise noted, each ion was prepared at the concentration of 1.0×10^{-3} M in distilled water and then diluted to a desired concentration. Li^+, Mg^{2+}, Mn^{2+}, Na^+, Pb^{2+}, Zn^{2+}, Ag^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Cu^{2+} and Ni^+ were prepared from their perchlorates. Cr^{3+} was prepared from $CrCl_3(H_2O)_6$. Pd^{2+}, Pt^{2+}, and Rh^{3+} were prepared from $PdCl_2$, $PtCl_2$, and $RuCl_3$, respectively: the metal chlorides were dissolved in dilute hydrochloric acid and then carefully adjusted to neutral with ammonia solution. Au^{3+} and Zr^{4+} were prepared from $AuCl_3(HCl)(H_2O)_4$ and $ZrCl_4$, respectively: $AuCl_3(HCl)(H_2O)_4$ or $ZrCl_4$ were dissolved in distilled water and then carefully adjusted to neutral with ammonia solution.

2.4. UV–Vis and fluorescence titration experiments

Unless otherwise noted, all the UV–Vis and fluorescence titration experiments were operated in 40:60 EtOH/H_2O (v/v) solutions. (λ_{ex} = 436 nm, scan speed = 240 nm/min, excitation slit = 5.0 nm, emission slit = 5.0 nm, and PMT voltage = 400 V). The pH of the solution was adjusted using $HClO_4$ or NaOH. The sum of the volumes of the metal ion solution introduced into the target system did not exceed 2% of the original volume to minimize the effect of volume changes on the fluorescence properties. All spectra were measured at room temperature.

2.5. Cell culture

The MARC-145 cell line and HeLa cell line were provided by the College of Veterinary Medicine, China Agricultural University. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2.

2.6. Cytotoxicity assay

MARC-145 cells were seeded in a 96-well plate and allowed to adhere for 24 h. Then the cells were incubated with different concentrations of **WM-S** (0, 10, 20, 40, 80 μM, containing 1% DMSO) for 24 h. Finally, the viability of MARC-145 cells in the culture environment of **WM-S** was analyzed using MTT cytotoxicity assays.

2.7. Fluorescence imaging experiments

HeLa cells were seeded in a 12-well plate and allowed to adhere for 24 h. Then the cells were treated with 10 mM solutions of the probe **WM-S** (1 μL; final concentration: 10 μM) dissolved in DMSO and incubated at 37 °C for 30 min. After removing the culture medium and washing twice with PBS, fluorescence images of cells were captured. DMEM was added to the cell culture, which was then treated with a 10 mM solution of Pd^{2+} (2 μL; final concentration: 20 μM) and incubated at 37 °C for an additional 15 min. After washing twice with PBS, fluorescence images of the cells were captured.

3. Result and discussion

3.1. Synthesis

The detailed synthetic route of compound **WM-S** is shown in Scheme 1. The reaction of coumarin aldehyde (compound **1**) and hydroxylamine hydrochloride in the solution of EtOH at room temperature gave the coumarin oxime **2**. Finally, compound **WM-S** was obtained by the reaction of compound **2** with 2-chloroethyl methyl sulfide in DMF at 100 °C. Compound **WM-S** was confirmed