Sensors and Actuators B 225 (2016) 572–578

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A carbazole-based mitochondria-targeted two-photon fluorescent probe for gold ions and its application in living cell imaging

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ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form
16 November 2015
Accepted 17 November 2015
Available online 23 November 2015

Keywords:
Two-photon fluorescent probe
Mitochondria
carbazole
Au^{3+}/Au^{+}
Bio-imaging

ABSTRACT

A carbazole-based two-photon fluorescent probe (**PyCM**) showed a remarkable fluorescence "turn-on" response to Au^{3+}/Au^{+} in aqueous solution with a large Stokes shift (119 nm). A good linear relationship of emission intensity at 539 nm against Au^{3+}/Au^{+} from 0 to 20 equiv. was observed, and the detection limits ($3\sigma/k$) were as low as 47 nM for Au^{3+} and 73 nM for Au^{+}, respectively. The fluorescence enhancement was attributed to the gold ions-induced C=N bond hydrolysis sensing mechanism, which is fully confirmed by the UV–vis absorption, fluorescence, IR, ^{1}H NMR titration and MALDI-TOF mass analysis. Bio-imaging study established that **PyCM** could be used to detect Au^{3+} in living cells under two-photon excitation with large two-photon absorption cross sections (1321 GM at 860 nm), little cytotoxicity and good biocompatibility. Meanwhile, standard co-staining experiments of **PyCM** and MitoTracker Red FM (co-localization coefficient: 0.85) revealed that **PyCM** was predominantly present in mitochondria.

1. Introduction

Mitochondria play a critical role in the regulation of apoptosis (programmed cell death) and in diseases characterized by abnormal apoptotic responses such as cancer, making them an attractive target for the design of new anticancer drugs [1–4]. Considering the gold lipophilic complex could be accumulated in mitochondria of cells, driven by the high mitochondrial membrane potential, several different classes of gold-based compounds (both Au(I) and Au(III) complexes) have attracted interest as potential antitumor agents, which have been evidenced for involving mitochondrial cell death pathways [5–10]. Due to the widespread application of gold species in mitochondrial targeted chemotherapeutics, it is highly desirable to develop efficient detection methods for gold species in mitochondria, which is of great importance for monitoring the gold mediated physiological processes in mitochondria, and advancing drug development in biomedicine.

Fluorescent probes have been evaluated as powerful tools to detect biological agents in recent years, due to their high sensitivity and selectivity, real-time detection and easiness of manipulating [11–15]. To date, fluorescent probes based on various fluorophore units including rhodamine [16–23], fluorescein [24–26], BODIPY [27–29], naphthalimide [30,31], coumarine [32–34], rhodamine-BODIPY coupling [35,36] dyes, have been developed to detect gold ions and some of them have realized the bio-imaging in living cells. However, those probes might encounter some problems, such as cytotoxicity, poor water solubility, small Stokes shift, low sensitivity and so on. Moreover, since mitochondria is the main site of gold complexes acting as anticancer drugs, monitoring gold species in mitochondria is particularly meaningful and valuable. Nevertheless, to the best of our knowledge, no report exists describing a mitochondria-targeted fluorescent probe for Au^{3+}/Au^{+}.

Recently, some mitochondria-targeted fluorescent probes realized the two-photon imaging and tracking of mitochondria through the use of two-photon microscopy (TPM) [37]. TPM, which employs two photons for the excitation in the near-infrared wavelength (ca. 700–1100 nm), provides an opportunity to overcome the problems originated from the single-photon fluorescence technology, such as excitation with short-wavelength light (ca. 350–550 nm), photobleaching, photo-damage, and cellular auto fluorescence [38–42]. Hence, there is a strong need to develop a two-photon fluorescent probe for monitoring Au^{3+}/Au^{+} in mitochondria, which emit two-photon-excited fluorescence (TPEF) at different wavelengths.

Herein, we developed a carbazole-based two-photon fluorescent probe [(3-((2-pyridinyl) hydrazono)methyl-6-(1-methyl-4-vinylpyridiumiodine)-9-ethyl-carbazole), **PyCM**] for Au^{3+}/Au^{+} detection with high selectivity, low detection limit, large Stokes

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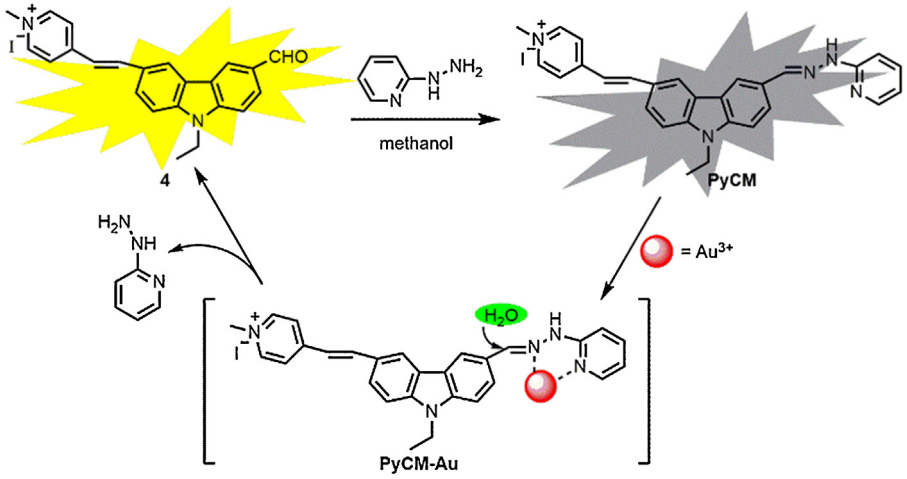
http://dx.doi.org/10.1016/j.snb.2015.11.081
0925-4005/



Scheme 1. Synthesis and Au^{3+}-induced hydrolysis of probe **PyCM**.

shift and two-photon absorption cross sections, which utilized the gold ions-induced C=N bond hydrolysis sensing mechanism as reported [23,26,28]. A novel donor-π-acceptor (D-π-A) two-photon fluorophore based on electron-donating carbazole and electron-withdrawing cationic pyridinium moiety was utilized. This excellent intermolecular charge transfer (ICT) system will guarantee the two-photon activity of the probe [37,43,44]. Meanwhile, as for **PyCM**, methyl pyridinium moiety as the mitochondria-targeted functional group was conjugated with the carbazole to realize the locating of mitochondria [44–46], which also enabled the probe with good water-solubility. In Scheme 1, we described that **PyCM** may give a remarkable fluorescence "turn-on" response to Au^{3+} in aqueous solution through Au^{3+}-induced hydrolysis to the corresponding aldehyde derivative [3-carbaldehyde-6-(1-methyl-4-vinylpyridium iodine)-9-ethyl-carbazole), compound **4**], which was fully proved by the UV–vis absorption, fluorescence, IR, ^{1}H NMR titration and MALDI-TOF mass analysis.

2. Experimental

2.1. Materials and equipments

All solvents were purchased and dried according to standard procedures before use. MitoTracker Red FM was purchased from Invitrogen (USA). ^{1}H NMR spectra were recorded on Avance II-400 MHz spectrometers and ^{13}C NMR spectra were recorded on 100 MHz spectrometers (Bruker). MS spectra were recorded on a MALDI-TOF/TOF 5800 mass spectrometer (AB Sciex). The detection of various ions were operated at pH 7.4, and maintained with PBS buffer. UV–vis absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu). Fluorescence spectra were obtained using a F-7000 Fluorescence spectrometer (Hitachi). The fluorescence quantum yields were detected by HORIBA FluoroMax-4P (HORIBA Jobin Yvon).

2.2. Synthesis

2.2.1. Synthesis of 3-((2-pyridinyl)hydrazono)methyl-6-(1-methyl-4-vinylpyridiumiodine)-9-ethyl -carbazole (*PyCM*)

A solution of compound **4** (0.234 g, 0.5 mmol), 2-hydrazinylpyridine (0.164 g, 1.5 mmol) and a drop of glacial acetic acid in methanol (15 mL) were stirred at 70 °C for about 3 h. The crude product was collected as powder. After recrystalization from methanol, **PyCM** was obtained as a yellow powder in 82% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 8.82 (d, J = 6.6 Hz, 2H), 8.71 (s, 1H), 8.48 (s, 1H), 8.22–8.18 (m, 4H), 8.12 (d, J = 3.8 Hz, 1H), 7.90–7.85 (m, 2H), 7.76 (d, J = 8.6 Hz, 1H), 7.73–7.63 (m, 2H), 7.57 (d, J = 16.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.77–6.75 (m, 1H), 4.51 (q, J = 6.8 Hz, 2H), 4.24 (s, 3H), 1.36 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.23, 152.99, 147.79, 144.75, 142.15, 141.32, 140.38, 139.86, 137.80, 127.43, 126.78, 126.56, 124.72, 124.68, 122.76, 122.47, 121.19, 120.12, 118.38, 114.55, 110.04, 106.21, 46.65, 37.43, 13.84. MS (MALDI-TOF): m/z: calcd. for $C_{28}H_{26}N_5^+$: 432.2183, found: 432.2516.

2.3. Preparation of the test solution

Solutions of **PyCM** (1 mM) were prepared in DMSO. Phosphate-buffered saline (PBS) buffer solution: 20 mM NaCl, Na_2HPO_4, $NaH_2PO_4{\cdot}2H_2O$, pH 7.4. The test solution of **PyCM** (10 μM) in CH_3CN/PBS buffer (2/8, v/v, 20 mM, pH 7.4) was prepared. The solutions of various testing species were prepared by dilution of the stock solution with PBS buffer solution (DMSO-PBS buffer (1:1, v/v) was used for Au^+ and Sb^{3+} due to the solubility problem). CN^- was prepared from n-Bu_4NCN. Various metal ions were prepared from AuCl, $AuCl_3$, $Ce(NO_3)_3{\cdot}6H_2O$, Cs_2CO_3, NaCl, $ZnCl_2$, $NiSO_4{\cdot}6H_2O$, $Cr(NO_3)_3$, $CoCl_2$, $MnCl_2{\cdot}4H_2O$, $CaCl_2$, $MgSO_4$, $BaCl_2$, KBr, $CuCl_2$, $FeCl_3$, $Al(NO_3)_3{\cdot}9H_2O$, $AgNO_3$, $SbCl_3$, $HgCl_2$, $SnCl_2$, $PbCl_2$.

2.4. Measurement of two-photon absorption cross sections (δ)

Two-photon excited fluorescence (TPEF) spectra were measured using femtosecond laser pulse and Ti: sapphire system (680–1080 nm, 80 MHz, 140 fs, Chameleon II) as the light source. All measurements were carried out in air at 20 °C. Two-photon absorption cross sections were measured using two-photon-induced fluorescence measurement technique and thus the cross sections can be calculated by means of the following equation [47]:

$$\delta = \delta_{ref}\frac{\Phi_{ref}}{\Phi}\frac{c_{ref}}{c}\frac{n_{ref}}{n}\frac{F}{F_{ref}}$$

Here, the subscripts *ref* stands for the reference molecule. δ is the two-photon absorption cross sections value, c is the concentration of solution, n is the refractive index of the solution, F is the TPEF integral intensities of the solution emitted at the exciting wavelength, and Φ is the fluorescence quantum yield. The two-photon absorption cross sections were tested with 1 mM **PyCM** saturated with 20 equiv. of Au^{3+} by using optically matching solutions of Rhodamine