Sensors and Actuators B 268 (2018) 264–269

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Acetaldehyde-modified-cystine as an enhanced fluorescent probe for intracellular glutathione imaging

Linlin Hu[a,b], Xing Wei[a], Jie Meng[a], Xiaoyan Wang[a], Xuwei Chen[a,*], Jianhua Wang[a,*]

[a] Research Center for Analytical Sciences, Department of Chemistry, College of Sciences, Northeastern University, Box 332, Shenyang 110819, China
[b] College of Pharmacy, Weifang Medical University, Weifang 261042, China

ARTICLE INFO

Article history:
Received 10 August 2017
Received in revised form 19 April 2018
Accepted 21 April 2018
Available online 22 April 2018

Keywords:
Acetaldehyde-modified-cystine
Fluorescent probe
Glutathione sensing
Intracellular imaging

ABSTRACT

The development of fluorescent probes for detection of glutathione (GSH) has attracted extensive attentions due to its close association with cellular functions and diseases. Herein, a fluorescence enhancement sensing system based on acetaldehyde-modified-cystine (AMC) is reported for the detection of thiols, providing detection limits of 36 and 52 μmol L^{-1} for GSH and cysteine (Cys) respectively. Importantly, the fluorescence response of AMC is highly selective toward thiols with respect to the coexisting species in real biological matrixes. Considering the fact that GSH levels in cancer cells is significantly higher than those of Cys and other reducing species, the fluorescence enhancing response of AMC in cancer cells is dominated by GSH. Therefore, this provides the possibility for selective intracellular GSH imaging in living cancer cells, i.e., HeLa cells in the present study.

1. Introduction

The intracellular thiols, e.g., glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), are components of many proteins and molecules in biological systems which plays crucial roles in human physiological processes [1–3]. GSH, the most abundant thiol compound in cells, maintains an adequate intracellular redox status, and alexipharmic free radicals and peroxides [4,5]. It is reported that the intracellular GSH concentration ranges from 1 to 10 mmol L^{-1} in different types of cells [6], and the level of GSH relates to various human diseases, e.g., cancer, HIV, and aging [4,7]. In consideration of significantly higher glutathione level in cancer cells compared with that in non-cancerous cells, the selective and sensitive detection of intracellular GSH is extremely valuable for the diagnosis of related diseases [8].

Currently, the development of specific imaging protocols for GSH in living cells has drawn extensive interest and a variety of sensing systems have been constructed for monitoring the changes of the intracellular GSH level. Besides the typical organic fluorophores [9], other fluorescent materials, e.g., carbon quantum dots [10], manganese dioxide (MnO_2)-nanosheet modified upconversion nanoparticles [11] and boron-dipyrromethene (BODIPY) modified Au nanoparticals [12], have been reported for GSH detection. Among those strategies, the optical sensing systems based on fluorescence probes exhibit apparent advantages over other strategies due to their easy operation and high sensitivity [13–17]. A malonitrile-functionalized tetraphenylethene (TPE) derivative probe is used to detect GSH over Cys or Hcy based on unique aggregation-induced emission (AIE) property [18]. Real-time imaging and quantification of glutathione dynamics in live cells are conducted based on reversible fluorescent probes [19]. However, in practice for intracellular GSH imaging in living cells, more effective probes with favorable selectivity are still highly required for the discrimination of GSH from coexisting biological molecules.

In the present study, a Schiff base compound, acetaldehyde-modified-cystine (AMC) is developed for the detection of thiols. AMC contains two Schiff bases (—C=N-) and one disulfide bond (—S—S—), and the autofluorescence is induced by the n-π* transition of the two —C=N— bonds [20]. The disulfide bond (—S—S—) is cleaved into thiol (-SH) in the presence of GSH due to thiol-disulfide exchange reaction and thus the fluorescence intensity is substantially enhanced, offering high sensitive and selective detection for GSH. The sensing system is successfully applied for selective visualizing the intracellular GSH in living cancer cells. In addition, AMC exhibits favorable biocompatibility as demonstrated by its low cytotoxicity, which can serve as a potential platform for intracellular GSH imaging in cancer cells.

* Corresponding authors.
E-mail addresses: chenxuwei@mail.neu.edu.cn (X. Chen), jianhuajrz@mail.neu.edu.cn (J. Wang).

https://doi.org/10.1016/j.snb.2018.04.114
0925-4005/

2. Experimental

2.1. Materials

Acetaldehyde, cystine, asparaginic acid, proline, isoleucine, DL-phenylalanine, lysine, arginine, histidine, glucose, sodium chloride (NaCl), potassium chloride (KCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$), ferric chloride ($FeCl_2$), zinc chloride ($ZnCl_2$), manganese(II) chloride ($MnCl_2$), magnesium sulfate ($MgSO_4$), bovine serum albumin (BSA), cysteine (Cys) and glutathione (GSH, reduced form) are purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). N-ethylmaleimide (NEM) is obtained from Aladdin Reagents Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumerma bromide (MTT) assay kit are received from Nanjing Key GEN Biotech Co., Ltd. (Nanjing, China). All reagents are of analytical reagent grade and directly used without further treatment or purification. Deionized water (DI water) of 18 MΩ cm is used throughout the experiments.

2.2. Preparations and characterizations

The Schiff base compound acetaldehyde-modified-cystine (AMC) is prepared by the aldol condensation of acetaldehyde and cystine in alkaline media as reported in the literature [21]. Briefly, 1.0 g of cystine is added into 50 mL of deionized water, and the mixture is adjusted to pH 10 with 0.1 mol L^{-1} NaOH solution. Then 2.0 mL of acetaldehyde is introduced into the solution drop-wisely and the reaction mixture is stirred for 4 h at room temperature. Afterwards, the reaction mixture is adjusted to pH 7 with 0.1 mol L^{-1} HCl solution to stop the reaction and the supernatant is collected after standing for overnight. The brown AMC powder is finally collected by freeze-drying under vacuum.

Fluorescence spectra of 10 mmol L^{-1} AMC in the range of 400–750 nm with $\lambda_{ex}/\lambda_{em}$ at 464/490 nm are recorded on an F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan) equipped with a 1 cm quartz cuvette. The slits of excitation and emission are both set at 5.0 nm, and the scan speed is set at 1200 nm min^{-1}. UV–vis absorption spectra are recorded in the range of 350–800 nm with a U-3900 UV–vis spectrophotometer (Hitachi High Technologies, Japan). FT-IR spectra is obtained by using a Nicolet-6700 FT-IR spectrophotometer (Thermo Electron, USA) within a range of 4000–500 cm^{-1}. Synergy H1 ELISA plate reader (BioTek, USA) is used to evaluate the cytotoxicity with HeLa cells by MTT assay. HeLa cells are cultured in a HERA Cell 150 incubator (Thermo Electron, USA), and the images are taken by a FV 1200 confocal fluorescent microscopy (Olympus, Japan). Nuclear magnetic resonance (NMR) spectroscopy including ^1H NMR, ^{13}C NMR are recorded on an Avance II NMR spectrometer (Bruker, Switzerland) at 600 MHz using D_2O as deuterated solvent.

Considering the fact that most cancer tissues have a more acidic environment (pH ~6.0) than normal tissues and the bloodstream (pH 7.4) [22], the variations of fluorescence of 1.0 mmol L^{-1} AMC at pH values close to that in physiological environment, e.g., pH 5.0, 6.5, 7.4, are investigated. Its fluorescence enhancement in the presence of GSH at three concentration levels, e.g., 0, 1.0 and 10.0 mmol L^{-1} at the same physiological pHs are also evaluated.

2.3. Selective fluorescence sensing of cellular GSH

Various amounts of GSH in phosphate buffer are mixed with 100 μL AMC (10 mmol L^{-1}) to provide final GSH concentrations of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 0.75, 1, 2, 5, 10 mmol L^{-1}. The mixtures are then incubated at 37 °C for 3 h and their fluorescence intensities are recorded. As a comparison, the fluorescence response of 1.0 mmol L^{-1} AMC to Cys is investigated at same experimental conditions.

The selectivity of AMC probe to thiols is evaluated by investigating the potential interferences of a series of substances, e.g., amino acids, metal cations, and some species present commonly in cancer cells. 1.0 mmol L^{-1} AMC probe is incubated in the presence of 1.0 mmol L^{-1} amino acids (including DL-phenylalanine, Lysine, Arginine, Proline, Isoleucine, Histidine, Asparaginic acid), 100 μmol L^{-1} metal cations (including K^+, Na^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}), 1.0 mmol L^{-1} glucose, GSH and Cys, 100 μg mL^{-1} bovine serum albumin (BSA), and the fluorescence is recorded.

Cys is the second most abundant thiol compound in cancer cells at the concentration of 30–200 μmol L^{-1}. For further evaluating the selectivity of AMC probe for GSH, the potential interference from Cys is investigated. Therefore, the fluorescence contribution of 200 μmol L^{-1} Cys on the fluorescence of GSH at various concentration levels, e.g., 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mmol L^{-1}, is investigated. Besides, the interfering effect of large amounts of Cys for fluorescence intensity is also recorded in the presence of Cys and GSH at the same concentration levels.

2.4. Cytotoxicity assay and intracellular GSH imaging

MTT assay is carried out for evaluating the biocompatibility of AMC. HeLa cells are cultured in 96-well plates with 1×10^4 cells per well for 24 h. The probe AMC with different concentrations in the culture medium, e.g., 0.25, 0.5, 1.0, 1.5, 2.0, 5.0 mmol L^{-1}, are added to each well and then incubated for 24 h. Thereafter, 20 μL of MTT (5 mg mL^{-1}) is introduced into each well and the cells are incubated at 37 °C for another 4 h. Then, 150 μL DMSO is afterwards introduced to replace the culture medium. After thoroughly mixing, the plates are read by a microplate reader and the absorbance at 570 nm is recorded. Each measurement is the average of six wells, and the viability of the untreated cells is set at 100% for comparison.

In order to further verify the effective sensing of GSH in physiological systems, the imaging capability of AMC probe for intracellular GSH is carried out in HeLa cells. In practice, HeLa cells are first cultured at a concentration of 10^4 cells per well for overnight in 22 × 22 mm glass coverslips in DMEM media at 37 °C in a 5% CO_2 incubator. The cultured cells are washed with phosphate buffer followed by treatment with 1.0 mmol L^{-1} AMC for 3 h. As the control experiment, the cells are pre-incubated with 500 μmol L^{-1} N-ethylmaleimide (NEM, a trapping reagent for a scavenger of thiols) for 30 min to scavenge the thiols in cells and followed by incubating with 1.0 mmol L^{-1} AMC in the culture medium for 3 h. Then the fluorescence images of both cells treated and untreated with NEM are obtained by a confocal laser scanning microscopy with $\lambda_{ex}/\lambda_{em}$ at 408/460–520 nm.

3. Results and discussion

3.1. Characterizations

The obtained acetaldehyde-modified-cystine (AMC) is formed by aldol condensation reaction, which contains one disulfide bond and two Schiff bases. To verify the structure of AMC, ^1H NMR and ^{13}C NMR of AMC are tested. ^1H NMR (600 Hz, D_2O), δ (ppm): 4.11(1H, t), 3.18-3.39 (2H, d), 1.90 (3H, d), 1.19 (H, m). ^{13}C NMR (600 Hz, D_2O), δ (ppm): 172.73, 53.53, 36.04, 23.51, 20.50.

The obtained AMC is further characterized by FT-IR spectra (as shown in Fig. 1). The stretching band at 1621 cm^{-1} and the broad band at 3392 cm^{-1} in the spectra of AMC and cystine indicated the presence of —C═O group and —OH group of amino acid respectively [21]. The stretching band at 1584 cm^{-1} are attributed to the formation of C═N—R (imine) between acetaldehyde and primary amine