Sensors and Actuators B 261 (2018) 210–217

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A lysosomes-targeted ratio fluorescent probe for real-time monitoring of micropolarity in cancer cells

Yafu Wang [a], Ge Wang [b], Kui Wang [a], Zaoxia Wang [a], Yuming Guo [a], Hua Zhang [a,*]

[a] Collaborative Innovation Centre of Henan Province for Green Manufacturing of Fine Chemicals, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, Henan Key Laboratory of Green Chemical Media and Reactions, School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang, Henan, 453007, PR China
[b] Xinxiang Medical University, 601 Jinsui Road, Hongqi Zone, Xinxiang, 453000, PR China

ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 8 January 2018
Accepted 13 January 2018

Keywords:
Lysosomal micropolarity
Ratiometric fluorescent probe
Cancer cell apoptosis
Real-time estimating
Ultrasensitive

ABSTRACT

Lysosomal micropolarity-change is one of the ultimate reflection factors of cell apoptosis and death in complex living organism. Thus, rapid and sensitive detecting of lysosomal micropolarity-change is beneficial for real-time estimating of cancer cell apoptosis. Many polarity-specific fluorescent probes have been reported, but lysosomal micropolarity-ultrasensitive ratiometric fluorescent probe with excellent two-photon property for real-time estimating the cancer cell apoptosis is scarce. In this work, an intramolecular charge transfer (ICT) two-photon ratiometric fluorescent probe (**DC**) was reported and evaluated to real-time estimate cancer cell apoptosis through ultrasensitive detection of lysosomal micro-polarity. **DC** appeared different fluorescence intensities at 580 and 600 nm in cancer cells, specifically. And its fluorescence change presented a linear change on ratio mode in the range of polarity (Δf = 0.2230–0.3274). The lowest detection of **DC** for polarity change can as low as Δf = 0.018 by fluorescent ratiometric signals. More importantly, based on the ultrasensitive changes of lysosomal micro-polarity, **DC** can be used to real-time estimate cancer cell apoptosis by microscopic imaging and flow cytometry. Thus, **DC** may have potential application varying from detecting of lysosomal micropolarity changes to real-time estimating of cancer cell apoptosis.

1. Introduction

Lysosomal polarity, an important biological parameter, is generated primarily by the localization of specific proteins to lysosome and its membrane [1]. In living cells, lysosomal polarity not only influences itself shape, structure and membrane permeability, but also determines the interaction activity of a plenty of biomacromolecules, such as hydrolytic enzymes. Furthermore, lysosomal polarity would affect many molecular events in living cells, including decomposition and phagocytic activity of hydrolytic enzymes, digesting large structures or cellular debris, autophagy, clearing out damaged structures and so on [1]. More importantly, the recent studies indicated that polarity-deficient could induce cell apoptosis and death [2–4]. Polarity micro-change at lysosomes has became the ultimate reflection factor of cell apoptosis and death in complex living organism [5,6]. Thus, to monitor lysosomal micro-polarity and to further estimate cell apoptosis degree, especially cancer cell apoptosis degree, are of great importance for the fundamental research cell biology and oncologic pathology. However, lysosomal polarity in living cells is an extremely complex biological parameter and will be interfered by factors from the living organism, such as dipolarity/polarizability, hydrogen bonding and so on [7,8], therefore, the method detection for it that needs to possess "very low or even zero interference" characteristics. Furthermore, lysosomal polarity locales in a very small organelle-lysosome (0.025–0.80 μm), which has higher requirements for imaging resolution [1]. Finally, but more importantly, lysosomal polarity appears regional differences, that is, it differs considerably from one region to another in lysosome [7,8]. Thus, existing imaging tools, which are mediated by lysosomal polarity micro-change to real-time estimate cancer cell apoptosis, is extremely difficult and necessitates the development of new effective imaging tools.

Fluorescent sensors combined with confocal microscopy imaging are the powerful and promising analysis tools for the real time monitoring many bio-endogenous molecules and environmental factors in living systems [9–12]. Therefore, polarity-sensitive fluorescent probes were selected as the research tools to detect

* Corresponding author.
E-mail address: zhanghua1106@163.com (H. Zhang).

https://doi.org/10.1016/j.snb.2018.01.132
0925-4005/

polarity in living organism. Up to now, several research groups have reported a series of polarity-sensitive probes [13–20], which have been applied to sensing of the polarity and the hydrophobic domains of biological macromolecules, such as proteins, nucleic acid and enzyme. Although a few of these probes are cell-permeable and appear specific subcellular organelle distributions [18–20], such as lysosome [19,20], there is no ratiometric fluorescent probe that show ultra-sensibility for the lysosomal micro-polarity. Moreover, None of these probes that detection for lysosomal micro-polarity have the characteristics of "low or even zero interference", this will undoubtedly reduce the accuracy of the detection. Furthermore, two-photon fluorescence probes have become the leading bioimaging tools based on the high penetration depth and "zero interference" of two-photon technology in living organism. Unfortunately, two-photon ratiometric fluorescent probe which was mediated by lysosomal polarity micro-change with "zero interference" to real-time estimate cancer cell apoptosis remains a "blank space", with no attempt reported.

Herein, we reported an intramolecular charge transfer (ICT) two-photon ratiometric fluorescent probe, (6-((2-aminoethyl)amino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile, **DC**), which could real-time detect lysosomal micro-polarity, and further estimate cancer cell apoptosis. The probe **DC** was used polarity-sensitive group as fluorophore [21]. Ethylenediamine group was as regulator which resulted in **DC** possessing appropriate p*K*a to target lysosome. All results from absorption spectrum, fluorescence spectrum, the outcome of whole-cell assays and flow cytometry demonstrated the rapid and quantitative response of **DC** to polarity with high selectivity and sensitivity. More importantly, according to the different fluorescence intensity at different wavelengths, this lysosomal micropolarity-ultrasensitive ratiometric fluorescent probe was used to real-time estimate cancer cell apoptosis. To the best of our knowledge, **DC** is the first lysosomal micropolarity-ultrasensitive ratiometric fluorescent probe with "zero interference" for real-time estimating of cancer cell apoptosis.

2. Material and methods

2.1. Probe DC preparation and basic characterization

The synthetic routes and basic characterization (NMR spectrum and mass spectrum) of **DC** and its intermediates were listed in Supporting Information. *A.R.* grade of reagents and solvents were purchased and used in all of the experiments. Purify compounds were purified by the column chromatographic with the fillers-silica gel (200–300 mesh). Newly prepared double distilled water was purified prepared by a Milli-Q system, which was used in all of the experiments. Under ESI positive or negative ion mode, ESI–MS (Bruker microToF II, Bruker Co., Switzerland) possessing the auto sampler operated in-line with a quantum triple quadruple instrument was carried out in the basic characterization of **DC** and its intermediates. Avance 400 MHz spectrometer (Bruker Co., Switzerland) was used to obtain NMR spectra.

2.2. Spectral determination

LysoTracker® Green DND was obtained from Invitrogen™ Chemical Co. (USA). LysoTracker® Green DND (1.0 mM, DMSO) and **DC** (1.0 mM DMSO) as stock solution were used in spectrographic determination, respectively. The determination method was described in Supporting Information. The results were obtained from five parallel tests.

2.3. Cell culture and staining with DC

HepG 2 (cancer cell line) and CHO (normal cell line), two kinds of cell lines were purchased from the Chinese Academy of Medical Sciences. The culture medium for this two cell lines are composed of phenol red-free Dulbecco's Modified Eagle's Medium (DMEM, WelGene), 10% fetal bovine serum (FBS; Gibco) and penicillin/streptomycin. HepG 2 and CHO cell lines were incubated in this kind of culture medium for 3 days in a CO_2 incubator at 37 °C. Before imaging, cells were seeded into a glass bottomed dish (MatTek, 35 mm dish with 20 mm well) for incubation 24 h in advance at 37 °C under 5 wt%/vol CO_2. And then, the live cells were incubated with a certain concentration of LysoTracker® Green DND (1.0 mM, DMSO) and **DC** (1.0 mM DMSO) at 37 °C under 5 wt%/vol CO_2.

2.4. One/two-photon fluorescence imaging and colocalization imaging

FV1200 spectral confocal multiphoton microscope (Olympus, Japan) was used to image cell on one-photon mode. This microscope combined with mode-locked titanium-sapphire laser (MaiTai, Spectra-Physics, USA) was used to obtain two-photon imaging in cells. The imaging method was described in Supporting Information. The results were obtained from five parallel tests.

2.5. Cancer cell apoptosis model building

HepG 2 cells were incubated by phenol red-free Dulbecco's Modified Eagle's Medium (DMEM, WelGene), 10% fetal bovine serum (FBS; Gibco) and penicillin/streptomycin. They were incubated in this kind of culture medium for 3 days in a CO_2 incubator at 37 °C. And then, the new culture medium containing 100 μM of tea polyphenols was replaced for incubation 24 h in advance at 37 °C under 5 wt%/vol CO_2. At last, the live cells were incubated with a certain concentration of **DC** (1.0 mM DMSO) at 37 °C under 5 wt%/vol CO_2.

2.6. Flow cytometry for estimating of cancer cell apoptosis by DC

After cancer cell apoptosis was established, their was incubated with **DC**. And then, those cell models (10,000 cells/500 μL) were made into dispersed PBS solution. And these were analyzed by flow cytometer (BD FACSCanto II, USA) on 488 nm. In data analysis, 10,000 cells were as the counting base. And BD FACSDiva software was used to analyze their average fluorescence intensity.

3. Results and discussion

3.1. Design of fluorescent probe

In this work, we reported a probe **DC** was an ideal two-photon fluorescent probe for the ultrasensitive detection of lysosomal micro-polarity. Through exploring the structure of this probe **DC**, 1-oxo-1*H*-phenalene-2,3-dicarbonitrile in molecule is a polarity-sensitive group [21], which result in probe appearing obviously response ability for micro-polarity. Lysosome, a typical organelle, contain a lot of acid hydrolases, which make it appearing acidity (pH ranging from 4.5 to 5.5). Consider this particular property of lysosomes, ethylenediamine group in **DC** could be as regulator which resulted in probe possessing appropriate p*K*a to target lysosome. Furthermore, due to the introduction of amino and cyano, the probe has an appropriate water-oil amphiphilicity which lead to the specific selectivity for cancer cells. The synthetized route of probe (**DC**) and the relevant intermediates were listed in Supplementary Scheme S1 (Scheme 1). **DC** and the relevant intermediates