Biosensors and Bioelectronics 68 (2015) 189–196

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Imaging of nucleolar RNA in living cells using a highly photostable deep-red fluorescent probe

Bingjiang Zhou [1], Weimin Liu [1], Hongyan Zhang, Jiasheng Wu, Sha Liu, Haitao Xu, Pengfei Wang *

Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, China

ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form
23 December 2014
Accepted 24 December 2014
Available online 26 December 2014

Keywords:
RNA
Nucleoli
Fluorescent imaging
Real-time

ABSTRACT

A new crescent-shape fluorescent probe (named here as **CP**) that selectively stains RNA in nucleoli of living cells is prepared. **CP** shows a deep-red emission (658 nm) and a large Stokes shift because of the introduction of rigid-conjugated coumarin moiety into the molecular structure. Cell imaging experiments indicate that **CP** can rapidly stain nucleoli in living cells by binding with nucleolar RNA, showing performance superior to commercially available nucleoli dye SYTO RNASelect in terms of high photostability and selectivity. More significantly, these excellent properties together with low cytotoxicity enable **CP** to monitor nucleolar RNA changes during mitosis, and after treating with anti-cancer drugs cisplatin, actinomycin D and α-amanitin. Thus, **CP** could be a potential tool for real-time, long-term visualization of the dynamic changes for nucleolar RNA and evaluation of the therapeutic effect for anti-cancer drugs that targeted RNA polymerase I (Pol I).

1. Introduction

RNA molecules are responsible for a wide range of functions in living cells, from physical conveyance and interpretation of genetic information, structural support for molecular machines, and regulation/silencing of gene expression, to essential catalytic roles (Prasanth and Spector, 2007; Storz et al., 2005). The ability to acquire complete spatial–temporal profiles of RNA synthesis, processing, and transport is therefore critical to understand cell function and behavior in health and disease conditions (Bao et al., 2009). In addition, most of the RNA in the nucleus is localized to the nucleolus, where is the key site of ribosomal RNA (rRNA) transcription, processing, and ribosome assembly (Yusupov et al., 2001). As one of the most powerful techniques for monitoring bimolecular in living systems, fluorescence imaging techniques are widely used to visualize morphological details of RNAs in cells. Those include microinjection of fluorescently tagged RNA molecules (Mhlanga et al., 2005; Wilkie and Davis, 2001), fluorescence in situ hybridization (Guo et al., 2012; Simon et al., 2010; Volpi and Bridger, 2008), molecular beacons (Chen and Tsourkas, 2009), and small-molecule fluorescent probes (Li and Chang, 2006; Liu et al., 2011; O'Connor et al., 2009). Among these, small-molecule fluorescent probes are the most practical tool for live cell imaging, because the good cellular permeability make it does not require fixation of cells which can disturb the cell membrane and organelles.

Considerable efforts have been exerted to develop small-molecule fluorescent probes for RNA imaging in living cells. However, in comparison with DNA probes, RNA probes, especially nucleolar RNA probes for live cell imaging are quite rare due to better affinity for DNA and poor nuclear membrane permeability. In 2006, Chang et al. first reported three selective RNA-binding styryl probes screened from 88 compounds through RNA assay and live cell imaging methods (Li and Chang, 2006). Recently, Song and Liu et al. also find other small-molecule probes for RNA in nucleoli and cytoplasm based on styryl dyes (Liu et al., 2014; Song et al., 2014). In addition, Stevens et al. designed an energy transfer probe for duplex RNA in cellular imaging (Stevens et al., 2008). Peng et al. reported a near-infrared RNA fluorescent probe for imaging living cells assisted by the macrocyclic molecule CB7 (Li et al., 2013). So far, the only commercially available nucleolar RNA probe is a green fluorescent dye known as "SYTO RNASelect", whose molecular structure has not been published to date. However, these reports on nucleolar RNA staining also have problems of poor photostability, high cytotoxicity, short emission wavelength ($<$ 600 nm), or poor cell permeability. Thus it is highly desirable to develop

* Corresponding author.
E-mail address: wangpf@mail.ipc.ac.cn (P. Wang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2014.12.055
0956-5663/

new fluorescent probes with excellent performance to monitor the changes of nucleolar RNA. The nucleolus is a subnuclear organelle that contains ribosomal RNA gene clusters and ribosome biogenesis factors.

In this study, a fluorescent probe **CP** was development by rigid hybridizing coumarin and pyronin moieties to form crescent-shape structure. The intramolecular charge transfer reservation and planarity amplification resulted in large Stokes shift and deep red emission. Then **CP** was found to be a potential fluorescent probes for imaging RNA distribution within the nucleolar. The photophysics properties of **CP** and its interaction with nucleic acid (NA) have been investigated through absorption spectroscopy and fluorescence spectroscopy. Moreover, we successfully used **CP** to study the details of nucleolar RNA dynamic changes during mitosis, as well as after treatment with inhibitor of RNA polymerase I (Pol I).

2. Experimental section

2.1. Materials and apparatus

All commercial chemicals were used without further purification. 7-amino-1-methyl-1,2,3,4-tetrahydroquinoline hydrochloride was purchased from HangZhou Trylead Chemical Technology Co., Ltd. *Escherichia coli* total RNA and Calf thymus (CT) DNA were obtained from Sigma-Aldrich. The stock solutions of **CP** were prepared in DMSO (1.0×10^{-2} M). All UV–vis and fluorescence spectra in this work were recorded using Hitachi U3900 and Hitachi F4600 fluorescence spectrometers, respectively. All measurements were carried out at room temperature. Water was purified by a Millipore filtration system. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were collected using a Bruker Advance 400 spectrometer with tetramethylsilane as an internal standard. Electrospray ionization high resolution mass spectra (ESI-HRMS) were obtained usi a Bruker Apex IV FTMS. Fluorescent images were acquired with a Nikon C1si laser scanning confocal microscope, equipped with a live-cell incubation chamber maintaining a humidified atmosphere of 37 °C and 5% CO_2. Images were processed by NIS-element analysis AR 4.13.00. Clear nucleoli of Hela cells amplification imaging were obtained by a Delta Vision OMX imaging system.

2.2. Synthesis

CP can be readily prepared by condensation reaction of 3-aldehyde-4-chlorocoumarin derivates and 3-aminophenol. Detailed synthetic routes and methods are shown in Scheme 1 and Supplementary information. **CP**: ^{1}H NMR (400 MHz, DMSO-d^6) δ 8.92 (s, 1H), 8.01 (d, J=9.5 Hz, 1H), 7.74 (s, 1H), 7.35 (d, J=9.3 Hz, 1H), 7.23 (s, 1H), 6.68 (s, 1H), 3.55 (s, 2H), 3.36 (s, 6H), 3.16 (s, 3H), 2.81 (s, 2H), 1.93 (d, J=10.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 161.61, 158.36, 158.14, 157.00, 156.54, 154.15, 145.09, 133.80, 122.81, 122.63, 116.27, 115.74, 105.39, 99.56, 97.34, 96.46, 50.97, 40.96, 26.34, 20.44. ESI-HRMS m/z calcd for $[C_{22}H_{21}N_2O_3]^+$ 361.15467, found 361.15508.

2.3. Determination of quantum yield

Fluorescence quantum yields (Φ_U) are measured using cresyl violet in methanol as the reference (Φ_R=0.54) based on the following equation:

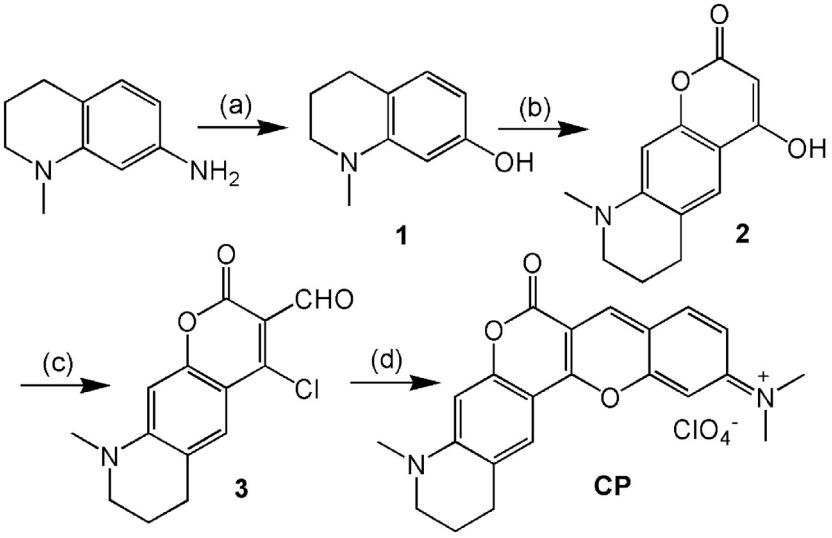
$$\Phi_U = \Phi_R\left(\frac{A_U}{A_R}\right)\left(\frac{{n_U}^2}{{n_R}^2}\right)$$

where A_U and A_R are the integrated areas under the corrected fluorescence spectra for the sample and reference, n_U and n_R are the refractive indexes of the sample and reference, respectively (Magde et al., 1979).

2.4. Cell culture and staining

Hela, A549 and L929 were gifted from the center of cells, Peking Union Medical College. L929, A549 or Hela cells were grown in RPM1640, McCoy's 5A or Dulbecco's modified Eagle's medium (DMEM) respectively, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and incubated under 5% CO_2 at 37 °C. Cells were seeded on confocal dish for imaging 12–24 h prior to conduction of experiment.

Below is a general procedure for labeling living cells. Cells were adhering to coverslips. **CP** was dissolved in DMSO to a final concentration. After added the appropriate of dye stock solution directly to the culture medium, cells were incubated for 20 min at 37 °C in 5% CO_2, and then imaged in dye medium at 37 °C in 5% CO_2 without wash. For colocalization experiments, **CP** was



Scheme 1. Synthesis of **CP**. Conditions: (a) 85% phosphoric acid, reflux, 24 h; (b) diphenyl malonate, toluene, reflux, 6 h; (c) phosphoroxychloride, 50 °C, 30 min; and (d) 3-aminophenol, acetic acid, 85 °C, 2 h, perchloric acid .