Biosensors and Bioelectronics 47 (2013) 566–573

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Direct visualization of the quadruplex structures in human chromosome using FRET: Application of quadruplex stabilizer and duplex-binding fluorophore

Yung-Chieh Chan [a], Jyun-Wei Chen [a], Sheng-Yuan Su [b], Cheng-Chung Chang [a,*]

[a] Graduate Institute of Biomedical Engineering, National Chung Hsing University, 250 Kuo Kuang Road, Taichung 402, Taiwan, ROC
[b] Department of Chemistry National Chung Hsing University, 250 Kuo Kuang Road, Taichung 402, Taiwan, ROC

ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
18 March 2013
Accepted 19 March 2013
Available online 29 March 2013

Keywords:
G-quadruplexes
Telomere
Chromosome
Fluorescent mapping
Fluorescence resonance energy transfer (FRET)

ABSTRACT

The G-quadruplex structures in the telomere of a chromosome can not only protect the internal chromosome sequences by preventing the improper activation of DNA-damage-response pathways but also become targets for cancer treatments. In this manuscript, we wish to prove the existence of G-quadruplex structure formation, rather than G-quadruplex sequence, in chromosome of human cancer cells. Based on our studies, the fluorescent mapping of G-quadruplex structures in the chromosome is possible with the combination of G-quadruplex targeting fluorophore (BMVC, 3, 6-bis-(1-methyl-4-vinylpyridinium)-carbazole diiodide) and duplex-binding fluorophores (Hoechst or propidium iodide). By means of an applicable incubation time between cell cycle period and proper staining procedure to the chromosome, FRET (fluorescence resonance energy transfer) between G-quadruplex targeting fluorophore and duplex-binding fluorophore can increase the signal contrast of the fluorescent color and the fluorescent mapping of quadruplex structures can be easily observed using fluorescence microscopy. These observations are further supported by basic spectral analysis, titration binding assay, gel electrophoresis binding competition assay and confocal microscopy.

1. Introduction

Since the demonstration of G-quadruplexes and G-tetraplexes telomeric DNA (Henderson et al., 1987; Sen and Gilbert, 1988; Sundquist and Klug, 1989; Williamson et al., 1989), the conformations and functions of G-quadruplexes have gained considerable research interest (Neidle and Parkinson, 2002; Dai et al., 2008) because this type of structure is a substrate of telomerase (Zahler et al., 1991) and quadruplex stabilizers could serve as antitumor agents (Mergny and Helene, 1998; Han and Hurley, 2000). Therefore, the efficient detection of G-quadruplexes, in addition to the stabilization of the G-quadruplex structure, would be very helpful for designing cancer drugs and for the diagnosis of various diseases (Song and Ren, 2010; He et al., 2006). In vertebrates, telomeres that consist of tandem G-rich repeating units, such as TTAGGG, are found in the tail ends of chromosomes. Additionally, the formation and stabilization of G-quadruplex require monovalent cations, in particular potassium and sodium (Sen and Gilbert, 1990; Hud and Plavec, 2006). That is, a G-rich sequence may adopt different structures in the presence of different cations (Patel et al., 2007; Burge et al., 2006; Parkinson et al., 2002; Phan et al., 2004; Ambrus et al., 2006; Dai et al., 2007; Lim et al., 2009; Heddi and Phan, 2011) and some researchers try to investigate the actual G-quadruplex structure using the spectral diversities of quadruplex-binding fluorophores (Yang et al., 2009, 2010; Chang et al., 2007). However, most of above-mentioned structures are resolved by spectral methods with an artificial sequence of DNA in vitro and there have been little discussions regarding whether these structures actually exist in vivo.

Fluorescence in situ hybridization (FISH) can be used to show the presence of the TTAGGG sequence (Meyne et al., 1989, 1990), but there is no direct evidence of the presence of the quadruplex structure in the cell, even in the chromosome. Recently, the presence of G-quadruplexes could be observed directly by in situ immunostaining and only the antibody specific for anti-parallel G-quadruplexes gave a strong signal (Hans and Daniela, 2009). However, the existence of each individual G-quadruplex structure that is mapped in the human telomere or chromosome is still questionable. To provide a more direct evidence of the existence of quadruplex structures, fluorescent signal detection with potential G-quadruplex targeting fluorophores can be used to make the structures visible under fluorescence microscopy. Here, we focus on the fluorescent image detection method because of its convenience and visibility. For this method, it is important to have a good G-quadruplex stabilizer or recognizer with fluorescent emission in the visible region.

* Corresponding author. Tel.: +886 4 22840733; fax: +886 4 22852422.
E-mail address: ccchang555@dragon.nchu.edu.tw (C.-C. Chang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.03.041

Based on the findings of our study, the organic small molecule BMVC can stabilize the $(TTAGGG)_4$ G-quadruplex structure of the human telomere (Chang et al., 2003a, 2003b and serve as an antitumor agent (Huang et al., 2008). In addition, significant increases in the fluorescence yield and the distinct fluorescence properties of BMVC bound to various DNA structures have allowed us to map the G-quadruplex structure in human metaphase chromosomes (Chang et al., 2004a, 2006a). These distinct properties have also allowed us to observe bright fluorescence spots from BMVC in the nucleus of cancer cells compared to the observed weak fluorescence of BMVC in normal cells (Chang et al., 2004b). Furthermore, a simple handheld device incorporating the BMVC molecule has been designed for low cost point-of-care screening of cancer cells (a) (Kang et al., 2007; Liao et al., 2009). Even though BMVC is a good quadruplex recognizing fluorophore, it still cannot make the fluorescent signal detectable using microscopy techniques because a large excess of duplex structures will interfere the monitoring of quadruplexes in the chromosome. Furthermore, the emission wavelength difference when BMVC binds with duplex and quadruplex is less than 20 nm. It means that the fluorescent color diversity between quadruple and duplex is unapparent when BMVC stains on the chromosome. Therefore, we developed a strategy of increasing the color contrast of the image using FRET to resolve this challenge in the molecular imaging of quadruplexes. This manuscript will illustrate why we chose Hoechst and propidium iodide (PI) as duplex-binding fluorophores and present the expected results with suitable staining procedure.

2. Experiment

2.1. Materials and apparatus

The BMVC molecule was synthesized and purified as described elsewhere (Chang et al., 2003b, 2005). The Colcemid, Hoechst, PI and cell culture medium were purchased from Invitrogen. Oligonucleotides LD (5′-GCGCAATTGCGC) and HT (5′- $(TTAGGGG)_4$) were purchased from Applied Biosystems. Tris-buffer solutions containing 10 mM Tris–HCl (pH 7.5) and 150 mM NaCl (KCl) were added with each DNA and heated to 90 °C for 5 min, cooled slowly to room temperature, and then stored at 4 °C for more than one day before use. The concentration of DNA was determined by measuring the absorbance at 260 nm and was adjusted to ~100 μM per unit structure. All the solvents employed were of spectrometric grade.

The fluorescence images were obtained using a Leica AF6000 fluorescence microscope equipped with a DFC310 FX digital color camera; the λ scanning and confocal images were obtained using a Leica TCS SP5 confocal fluorescence microscope. The excitation source for the compounds was a 405 nm diode laser or 488 nm argon laser. Fluorescence photographs were taken throughout the relevant ranges using photomultiplier tubes (PMT)

2.2. Methods

2.2.1. Page

To perform the binding assay, certain concentrations of BMVC, Hoechst and/or PI were incubated with 20 μM of DNA HT or LD in tris-buffer solution for 10 min at room temperature. The reaction products were analyzed using 20% polyacrylamide gels. The gels were run at 100 V/cm for 90 min at 4 °C. The fluorescent gels were photographed using a digital camera under 365 nm UV light from handheld UV lamps, and the UV shadowing gel was observed under 254 nm UV light using aluminium-backed flexible silica TLC plates as the background.

2.2.2. Stock cell cultures

MCF7 human breast cancer cells and HeLa human cervical cancer cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) in a 5% CO_2 incubator at 37 °C for 24–36 h prior to treatment with the chemical compounds.

2.2.3. BMVC labeling of the metaphase chromosomes

The cells were arrested at metaphase of the cell cycle by treating with 0.1 μg/mL Colcemid for 4 h; then they were incubated with BMVC (10 μM) for 1 h, followed by gentle mixing before placing the culture back into the incubator. After the incubation, the medium was removed from the dish, and Trypsin was added to transfer the suspended cells to a 15 ml conical tube. Following the standard protocol, the sample was centrifuged (1000 rpm, 10 min, 4 °C) and the pellet was gently re-suspended by adding 0.075 M KCl hypotonic buffer followed by standing it for 20 min and then centrifuging it (1000 rpm, 10 min, 4 °C) again. Finally, the arrested cells were stored by mixing with Carnoy's Fixative solution (methanol/acetic acid (3:1)). Before spreading the chromosome, the cells were treated with Hoechst (1 μM) or propidium iodide (3 μM); then a 15–20 μL aliquot of the cell suspension was dropped onto a slightly humidified slide which was placed on a hot metal plate to evaporate the fixatives. The sample was washed before the imaging study.

3. Result and discussion

3.1. Basic spectroscopic properties and FRET illustration

The UV–visible absorption and fluorescence spectra of the compounds Hoechst, BMVC and PI were measured in DNA containing tris-buffer, as shown in Fig. S1. Basically, the emission intensities of free forms of these three compounds were very low, especially in aqueous solution. It is interesting that emission intensities of three compounds all enhanced once binding with duplex LD, while only the fluorescence intensity of BMVC enhanced in the presence of quadruplex HT. It is considered that the DNA-binding spectra of these compounds may predict or mimic the spectra diversity of these compounds with respect to chromosome environment. Thus, taking advantage of the data of Fig. S1, we present the spectra responses of compounds toward DNA in tris-buffer. Fig. 1 shows that there are virtual overlaps between emission spectra of the Hoechst and absorption spectra of BMVC; and between emission spectra of the BMVC and absorption spectra of PI.

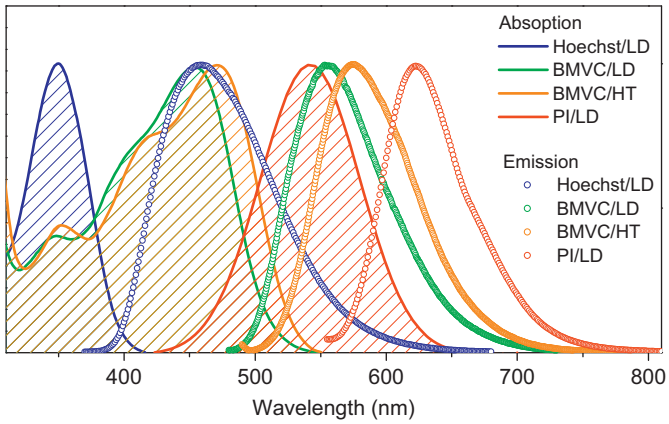

Fig. 1. FRET illustration between Hoechst (donor) and BMVC (acceptor) and between BMVC (donor) and PI (acceptor). Spectra were recorded in tris-buffer following Fig. S1.