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Determination of cell metabolite $VEGF_{165}$ and dynamic analysis of protein–DNA interactions by combination of microfluidic technique and luminescent switch-on probe

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ABSTRACT

In this paper, we rationally design a novel G-quadruplex-selective luminescent iridium (III) complex for rapid detection of oligonucleotide and $VEGF_{165}$ in microfluidics. This new probe is applied as a convenient biosensor for label-free quantitative analysis of $VEGF_{165}$ protein from cell metabolism, as well as for studying the kinetics of the aptamer–protein interaction combination with a microfluidic platform. As a result, we have successfully established a quantitative analysis of $VEGF_{165}$ from cell metabolism. Furthermore, based on the principles of hydrodynamic focusing and diffusive mixing, different transient states during kinetics process were monitored and recorded. Thus, the combination of microfluidic technique and G-quadruplex luminescent probe will be potentially applied in the studies of intramolecular interactions and molecule recognition in the future.

1. Introduction

Proteins serve as the molecular “workers” of cells that play important roles for carrying signals, chemical messengers, and regulating the functions in cells. Detection and quantification of proteins during cellular metabolism will be beneficial to understand the cell culture conditions and reflect the resultant effects on the productions (Burleigh et al., 2011; Cairns et al., 2011; Gambhir et al., 2003), which will promote the understanding of cancer disease-related biological processes (Ghesquière et al., 2014; Jain et al., 2012; Oladipupo et al., 2011; Papadopoulos et al., 2012), and also reveal the intracellular biochemical reaction rates (DeBerardinis and Thompson 2012; Paige et al., 2012; Wu et al., 2013; Papadopoulos et al., 2012). Recently, in-depth insights into cell metabolism become an important tool in the disease-marker discovery in the basic and applied biomedical researches (Chen et al., 2012; Xu et al., 2014; Ning et al., 2014). In general, traditional approaches for the determination of protein and nucleic acid interactions are commonly implemented by gel retardation, DNASE1 foot-printing, methylation interference, chromatin immune precipitation. Most of them are tedious, time-consuming, and high cost. An efficient and simple strategy for rapid and sensitive detection of cancer-related proteins is, thus, an urgently required.

Nucleic acid aptamers are single-stranded oligonucleotides from DNA/RNA libraries, which can bind specific molecular targets (Freeman et al., 2012; Iliuk et al., 2011). Due to their high affinity and specificity, aptamers have been most widely used for disease diagnosis, cell therapy, drug delivery and biosensors. More importantly, functional nucleic acid (FNA), which is aptamer assembled with DNAzyme (Aleman-Garcia et al., 2014; Nakayama and Sintim 2009) is available for catalyzing chemical reactions by binding the targets. Meanwhile, because the long-lived phosphorescence allowing for distinguishing fluorescent environment, large Stokes shifts can reduce self-quenching, and modular synthesis that can easily change the photophysical and molecular recognition properties without labour-intensive synthetic protocols (Leung et al., 2013; Ma et al., 2014), photoluminescent iridium (III) complex has attracted more and more interests in luminescence signaling and sensory applications. We report herein the synthesis and application of a novel G-quadruplex-selective

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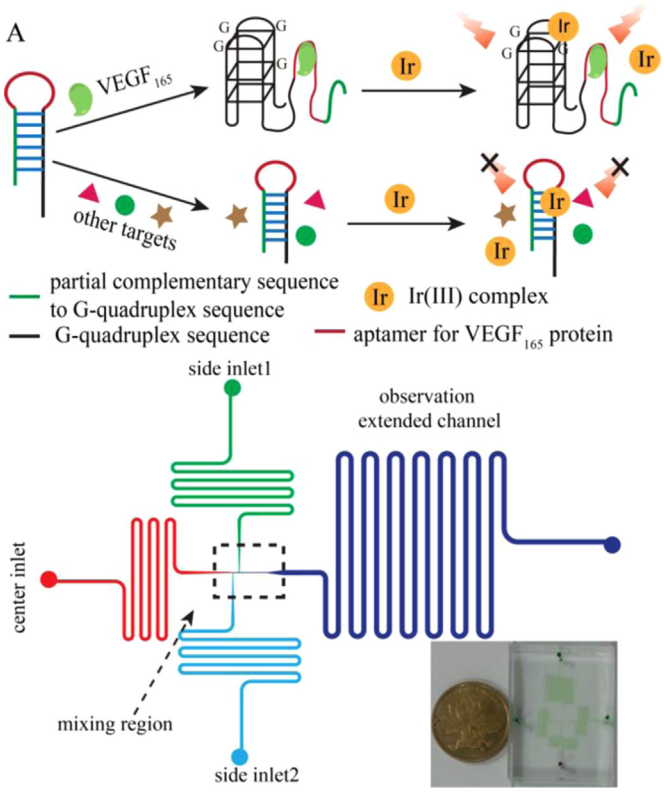

Fig. 1. G-quadruplex probe **1** for $VEGF_{165}$ assay in the microchip. (A) Schematic illustration of $VEGF_{165}$ assay based on G-quadruplex probe **1**. (B) The profile of microfluidic mixing device. The center inlet was applied to introduce $VEGF_{165}$ solution. The side inlet2 was used to introduce DNA solution, while complex **1** was injected from side inlet1. Solutions can be rapidly mixed in the mixing region. Extended observing channel prolongs the observation time up to minutes.

luminescent iridium (III) complex **1** for label-free, rapid and sensitive determination of $VEGF_{165}$ from cell metabolite. The mechanism of this assay is illustrated in Fig. 1, and the structure of iridium (III) complex **1** in Fig. 2A. Nowadays, quantitative investigation of the interplay of protein and nucleic acid shows great promises for revealing the potential mechanism of cellular signaling process. For example, quantitative investigation of the interplay of protein and nucleic acid shows great promises for revealing the potential mechanism of cellular signaling process. For these reasons, monitoring the interactions of protein and nucleic acid will facilitate to understand the dynamic equilibrium of protein-nucleic acid complex or protein folding pathways, and to elucidate the transitions occurring between different conformational states (Hwang and Myong 2014; Konishi et al., 2012; Schuler and Hofmann 2013). Meanwhile, because protein-nucleic acid interaction occurs at the moment when protein molecules promote a DNA/RNA conformational transition (Hofmann, et al., 2010; Lipman et al., 2003), dynamic resolution of the instruments is required on milli-/micro- second scale resolution (Vahidi, et al., 2013; Zhang et al., 2014). It is a huge challenge by the existing method. Recently, microfluidic mixing is emerged as an excellent method for observing transient states of protein and nucleic acid complexes (Gambin, et al., 2010; Johnson, et al., 2002; Xia, et al., 2005; Wunderlich et al., 2013) due to the following advantages: (i) eliminating turbulence and enhancing laminar and steady fluid flow in microchannel, providing the possibility of building a mathematical model for complex geometries; (ii) precisely controlling flow rates at a short diffusion time allows them to achieve the accurate results within milli-/micro-second scale and little effect on surrounding environments; (iii) easily recording the reaction process by emplacing different observation positions along the microchannel; (IV) only an extremely small sample volume (microliters) is required for each assay, resulting in minimizing reagent and sample consumption. However, despite its high scale resolution and its broad dynamic range, the stability of the system is limited especially in the multiplex inlets. In this context, the U sharp channels are designed at nearby the inlets. The adding U-sharp channels alter the pressure of the system, resulting in greatly improving the stability of the system.

Vascular endothelial growth factor ($VEGF_{165}$), a kind of glycolated protein, is a crucial angiogenic mitogen over-expressed in the tumor cells, and can induce their migration, excessive proliferation, invasion and metabolism in the body (Choi et al., 2013; McCarthy, 2012; Saharinen et al., 2011). Moreover, various kinds of $VEGF_{165}$ aptasensor have been developed (Cho et al., 2012; Freeman et al., 2012; Iliuk et al., 2011; Kwon et al., 2012). As a proof-of-concept, $VEGF_{165}$ is selected as a model analyte of glycolated protein. The cell metabolite $VEGF_{165}$ was analyzed based on a new synthesized iridium (III) complex and a fragment of FNA. The iridium (III) complex was a G-quadruplex-selective luminescent probe. The FNA fragment is hairpin structure including $VEGF_{165}$ aptamer, G-quadruplex sequence and its complementary sequence. The usage of this aptasensor can specifically detect $VEGF_{165}$ with low background because the hairpin structure cannot bind to iridium (III) complex, and hairpin structure of fragment is not easy to degrade. The high luminescent signal can be only recorded when it is in the present of $VEGF_{165}$ because the hairpin structure was destroyed. Furthermore, we designed a microfluidic device for monitoring protein–nucleic acid interactions. The application of U-sharp channels greatly improve the stability in the system, and the frequency of every transients can be recorded combination with iridium (III) complex 1. The results showed that our designed device can not only provide a highly sensitive system for kinetic study of protein-DNA complexes especially in cases where reagents or samples are in limited supply, but also for cell metabolites analysis.

2. Experimental section

2.1. Cell culture

CaSki cell line was purchased from Cancer Institute & Hospital Chinese Academy of Medical Science (Beijing, China). CaSki cells were cultured in DMEM media supplemented with 1% fetal bovine serum (FBS), 100 μg/mL penicillin, and 100 μg/mL streptomycin. CaSki cells were also cultured with the final concentration 10 μg/mL of paclitaxel. Cells were maintained in a humidified atmosphere with 5% CO_2 at 37 °C.

2.2. Iridium complex 1 synthesis

The complex **1** were prepared according to literature methods (Ma et al., 2014), but the ligand is different. All complexes are characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry (HRMS). Complex **1**. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.44–8.42 (d, J=8.0 Hz, 2H), 8.15 (s, 2H), 8.09–8.05 (m, 4H), 7.88 (s, 2H), 7.64 (s, 10H), 7.18–7.14 (t, J=8.0 Hz, 2H), 6.76-6.70 (t, J=8.0 Hz, 2H), 5.78-5.76 (d, J=8.0 Hz, 2H),2.38 (s, 6H); ^{13}C NMR (400 MHz, Acetone-d6) δ 164.8, 164.0, 163.9, 161.4, 159.8, 153.7, 153.6, 151.2, 150.9, 148.6, 139.7, 135.8, 129.7, 129.6, 129.0, 128.5, 128.0, 127.8, 125.0, 123.8, 123.7, 123.5, 113.7, 113.5, 98.6, 98.3, 98.1, 27.2; HRMS: Calcd. for $C_{48}H_{32}F_4IrN_4$ $[M–PF_6]^+$: 933.2192, found: 933.2224; elemental anal. ($C_{48}H_{32}N_4IrPF_{10}$) C, H, N: calcd: 53.48, 2.99, 5.2; found: 53.23, 3.07, 5.25.