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Highly sensitive detection of hepatitis C virus DNA by using a one-donor-four-acceptors FRET probe

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ABSTRACT

Sensitive detection of Hepatitis C virus (HCV) DNA is critical to treat and prevent the propagation of hepatitis C diseases. Herein a FRET probe of One-Donor-Multi-Acceptors (D–mA) assembly is designed through the formation of triple-helix DNA structure, which can greatly reduce the background of FRET system so as to greatly increase the detection sensitivity of hepatitis C virus DNA with the limit of detection (3σ) being 24.57 nmol/L, making the detection of clinic samples successful and showing that the newly developed FRET probe of D–mA assembly could be applied for the early diagnosis and treatment of hepatitis C disease.

1. Introduction

Hepatitis C is a liver disease induced by hepatitis C virus (HVC), which can cause acute or chronic hepatitis infections. This chronic hepatitis will deteriorate into cirrhosis of the liver or even worse into liver cancer in a few years if not treated on time [1]. According to WTO, an estimated 150 million people have chronic hepatitis C infection globally, and approximately 71 million people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma [2–4]. Since an effective vaccination against HVC is still under development, early diagnosis of hepatitis C is critical to treat and prevent the propagation of this infectious disease [5]. Therefore it's important to develop methods combining with sensitive means of detection and qualification.

Now, both enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR) detections are the mainly applied methods for the clinic detection of HCV [6–9]. Although accurate, these methods to diagnose HCV infection are time-consuming since it takes a long time for most of them to generate antibodies, and it also needs enzymes which are cost and could be easily denatured and digested by proteases. Thus, effective approaches for HCV detection and quantification in early stage are urgently needed.

Föster resonance energy transfer (FRET), occurred between two photoluminescent (PL) chromophores with the absorption spectra of one chromophore, commonly named as acceptor, overlapping the emission spectra of another chromophore, commonly named as donor, through the interaction such as dipole-dipole, has found wide applications since it can supply long wavelength shift and low signal to noise ratio. FRET as a homogeneous analytical detection technique has the advantages of high sensitivity and selectivity, easy operation and so on, and thus was applied for analytical detection [10–12] and biological imaging [13–19].

Nucleic acid FRET (naFRET) probes, which signal the relative state changes of both the donor and the acceptor during the FRET process, are easily constructed since the oligonucleotides with the backbones labelled with one or several chromophores including donor fluorophores and acceptor fluorophores, have been used through varieties of hybridization reactions [20–25]. However, most naFRET probes reported up to now consist of one-donor-one-acceptor assembly, limiting the energy transfer efficiency (E_{FRET}). Probably owing to the weak dipole-dipole interactions that makes the energy of donors can't be transferred to the acceptors effectively, the as-resulting high background signal of the FRET probe of one-donor-one-acceptor assembly is not benefit to the sensitive detection of target [26]. On the contrary, multiple acceptors FRET probes can avoid this problem since the multiple acceptors might individually interact with the donors in dipole-dipole mode to collect the excitation energy from the donor with independent FRET rates, $k_{D\rightarrow An}$, resulting in a total transfer rate of $k_T = \Sigma_n k_{D\rightarrow An}$ Therefore, design of one-donor-multiple-acceptors (D-mA) can greatly increase the overall probability of energy transfer, and can amplify the FRET efficiency also in that way according to equation [1]:

$$E_n = \frac{k_T}{k_T + \frac{1}{\tau_{D(0)}}} = \frac{\sum_n k_{D\rightarrow A_n}}{\sum_n k_{D\rightarrow A_n} + \frac{1}{\tau_{D(0)}}} \quad (1)$$

Wherein $\tau_{D(0)}$ is fluorescence lifetime of the donor in the absence of

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acceptors. Theoretically, if all acceptors are located in one position and work independently, the FRET efficiency (*En*) simplifies to equation [2]:

$$E_n \approx \frac{n \bullet k_{D\to A}}{n \bullet k_{D\to A} + \frac{1}{\tau_{D(0)}}} = \frac{1}{1+\frac{1}{n}\left(\frac{R_{D\to A}}{R_{0,D\to A}}\right)^6} \quad (2)$$

It can be seen from the above formulas that the energy transfer efficiency (*En*) gets increased with the increase of the number of receptors, and the background signal originated from the donor then gets decreased. In such case, D–mA systems could remarkably enhance the FRET efficiency as compared to the ordinary system of one-donor-one-acceptor assembly (D–A) [27], greatly reducing the background and increasing the detection sensitivity if D–mA FRET probes could be properly developed.

It is known that polypurine–polypyrimidine sequences can assemble into triple helical DNA structures under acidic conditions, forming T-A•T and C-G•C^+ nucleobase triplets upon protonation of cytosine bases through Watson-Crick and Hoogsteen interactions [28–32]. For the formation of triple-helix structure, sensing platforms and molecular switches thus have been assembled, and found wide applications for lots of targets such as nucleic acids [33–37], metal ions [38,39], pH value [40,41], enzymatic activities [42] and so on. The formation of triple-helix structure, on the other hand, supplies us a good chance to modify the oligonucleotides with several chromophores simultaneously, making it advantageous to the construction of D–mA naFRET probes.

Here we successfully designed a one-donor-four-acceptors (D–4 A) FRET probe through the formation of triple-helix DNA structure. The D-4A FRET probe was formed by incubating the mixture of DNA A1, A2 and D1. Then the fluorescence of donor (FAM) was quenched because of FRET from donor to acceptors (BHQ1) when the probe was formed, and it was recovered due to disintegration of probe structure when the HCV DNA was added.

2. Material and methods

2.1. Materials

The oligonucleotides along with Labelled DNA were synthesized and HPLC-purified by Sangon Biological Engineering Technology & Co. Ltd. (Shanghai,China). All the DNA stock solution was prepared with ultrapure water (18.2 MΩ) and were showed in Table S1. Agarose [Gel Strength (1%) 750 gr/cm^2, gelling temperature: 37 ± 1.5 °C] was purchased from Hongkong Gene Co. Ltd., Phosphate buffer solution (PBS) (10 mM) was used in the whole experimental process. All solutions were prepared or diluted with Milli-Q water. Haemoglobin and albumin were purchased from Huaimeike Biological Technology & Co. Ltd. (Beijing, China) All other chemical reagents were of analytical reagent grade.

2.2. Apparatus

Fluorescence emission spectra measurement was performed on an F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Solutions were blended using a vortex mixer QL-901 (Haimen, China) before each measurement. An Eppendorf ThermoMixer C (Eppendorf, Germany) was used control the rate of temperature rise and fall as well as the incubation time during DNA incubation and pH value was measured by pH 700 pH meter (Eutech, Shanghai, China).

2.3. Preparation of D–MA probe

DNA A1, A-2 and D1 were added to the 10 mM sodium phosphate buffer containing 10 mM NaCl, 2.5 mM MgCl2, and 25 mM KCl, pH 6.6. After incubate at 95 °C for 10 min, the temperature of the mixture solution was programed cooled at the rate of 3 °C/min until to 35 °C. Then the solution was incubated for another 40 min at 35 °C. Finally the D–MA probe was formed. The final concentration of all DNA mentioned above was 200 nM.

2.4. Fluorescence quenching efficiency

Fluorescence quenching efficiency of donor ($E_{Q)}$ was calculated by the equation [3]:

$$E_Q = \frac{F_0 - F}{F_0} \quad (3)$$

Wherein, F_0 is the fluorescence intensity of donor before the probe was formed, and *F* is that after the probe was formed.

2.5. Agarose gel electrophoresis

In the gel electrophoresis assay, a sample containing 10 μL of each reaction sample, 3 μL 6× loading buffer, and 2 μL of SYBR Gold was subjected to the 2% agarose gel electrophoresis. For the D–4 A probe preparation, 1 μM (A1) and 1 μM (A2) were mixed with 1 μM (D1) in sodium phosphate buffer containing 10 mM NaCl, 2.5 mM MgCl2, and 25 mM KCl, pH 6.6. After incubated at 95 °C for 10 min, the temperature of the mixture solution was programed cooled at the rate of 3 °C/min until to 35 °C. Then the solution was incubated for another 40 min at 35 °C. All the gels were prepared using a 1 × TBE buffer and were run at 110 V for 40 min.

2.6. Selectivity of HCV DNA detection

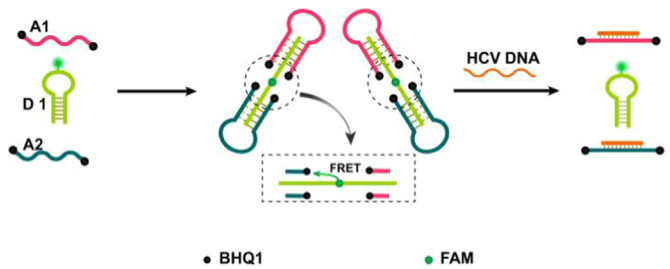
The same amount of HCV DNA, mis-1, mis-3, and mis-5 were added to 200 nM as-prepared D-4A FRET probe solution, and the fluorescence intensity of the solution with 200 nM HCV DNA, mis-1, mis-3, and mis-5 in 10 mM PBS buffer (pH 6.6) was measured after incubation at 35 °C for 40 min.

3. Results and discussion

3.1. Design of D–4A FRET probe

As shown in Scheme 1, the D–4A FRET probe assembled by three ssDNA strands, two of which were target specific aptamer sequences flanked by two arm segments, which are simultaneously labelled with acceptors (BHQ-1) at both 5′- and 3′- ends, and the other is donor (FAM)-labelled hairpin structure.

When the three sequences of ssDNA strands were mixed, heated and incubated, the arm segments of two aptamers' are bound with the loop sequence of FAM-labelled DNA (donor, D1) by Watson-Crick and Hoogsteen base pairings, respectively. As a result, the D–4A FRET probe was formed, leading to the occurrence of FRET from one FAM molecule to four BHQ-1 molecules, and thus the fluorescence of the donor (FAM) got efficiently quenched as the consequence. The as-prepared D–4A



Scheme 1. Illustration of synthesis process of one-donor-four-acceptors (D–4A) FRET probe and the highly selective and sensitive detection of HCV DNA detection.