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Detection of microRNA using enzyme-assisted amplifying and DNA-templated silver nanoclusters signal-off fluorescence bioassay

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ABSTRACT

A Simple and fast analysis strategy of fluorescence quenching based on DNA-templated silver nanoclusters was developed for detection of miR-122 related to diseases such as human liver. We used Exo III to cleave the silver cluster template and assist in the DNA-RNA complex cycle. When the target is absent, the silver cluster template remains intact, and DNA-AgNCs are generated under the action of $AgNO_3/NaBH_4$, producing a strong background fluorescence signal. Once the target is added, the site of the Exo III occurs after a series of hybridization cycles, the Exo III acts, the template DNA is continuously hydrolyzed, and the fluorescence intensity of the system is significantly reduced. By comparing the changes in the fluorescence signal, we found that this strategy has good sensitivity and the detection limit is as low as 84.0 pM. The strategy also has excellent discriminating ability and good selectivity, it can provide a persuasive reference for the early diagnosis of liver cancer and hepatitis.

1. Introduction

MicroRNAs (miRNAs) are endogenous noncoding short RNAs with lengths of 18–25 nucleotides (nt) [1–5], in recent years, as a biomarker and potential therapeutic target of many diseases [6–9], it has received tremendous attention. So far, many analytical methods such as electrochemical methods [5,10–13], electrochemiluminescence [14], fluorescence polarization analysis [6,15], surface-enhanced Raman spectrum [16–18], colorimetry analysis [19], magnetic relaxation time response analysis [20] and fluorescence analysis [21–23] et al., have been successfully applied to the analysis and detection of miRNAs. Among them, the fluorescence-based detection system is widely used in biochemical analysis due to its simple operation, stable signal response, high sensitivity and low cost [24–26].

Since accurate detection of miRNAs is important for clinical diagnosis and pathogenesis research, and there are still great challenges to sensitive and selective detection of miRNAs, so it is of great necessity for us to develop simple, fast and sensitive miRNA analysis platforms [27]. Recently, a majority of amplification strategies such as hybridization chain reaction (HCR) [28], rolling circle amplification (RCA) [29,30], real-time polymerase chain reaction (RT-PCR) [31], catalyzed hairpin assembly (CHA) [32], and the enzyme-based cyclic amplification [33] have been successfully applied for miRNAs to greatly improve the sensitivity. Among them, the enzyme-based cyclic amplification has become a widely used strategy for the analysis of miRNAs due to its outstanding amplifying function and can be used for homogeneous liquids [34]. Even though these strategies greatly improved the sensitivity of target detection, but they still have some drawbacks such as laborious procedure, time-consuming, involving of labelling and high cost. Therefore, the development of a high-speed, low-cost and labeled-free miRNA detection method is still necessary.

Metallic nanoclusters as collections are composed of several to dozens of atoms and have the characteristics of facile synthesis and tunable fluorescence emission DNA-templated Silver nanoclusters [35–37], as promising nanomaterials, have fascinating features such as high quantum yield, good water solubility, low toxicity and excellent biocompatibility [38–40], it does not need any additional fluorophores and quenchers to easily enhance the fluorescence signal. Based on these performances, DNA-AgNCs have been successfully used for various biomedical applications [40]. For instance, Yuan et al. achieved high-sensitivity detection of miRNA by in situ electrochemical generation of DNA-stabilized silver nanoclusters using target cycle synchronous RCA products [30]. Zhou et al. developed a novel dual fluorescence temperature-sensitive chameleon DNA-templated silver nanocluster pair for intracellular temperature measurement [39]. Zhang et al. utilized AgNCs based on fluorescence enhancement of G-rich sequences for miRNA imaging in cancer cells [41]. These methods have improved the sensitivity of analysis of miRNA to some extent.

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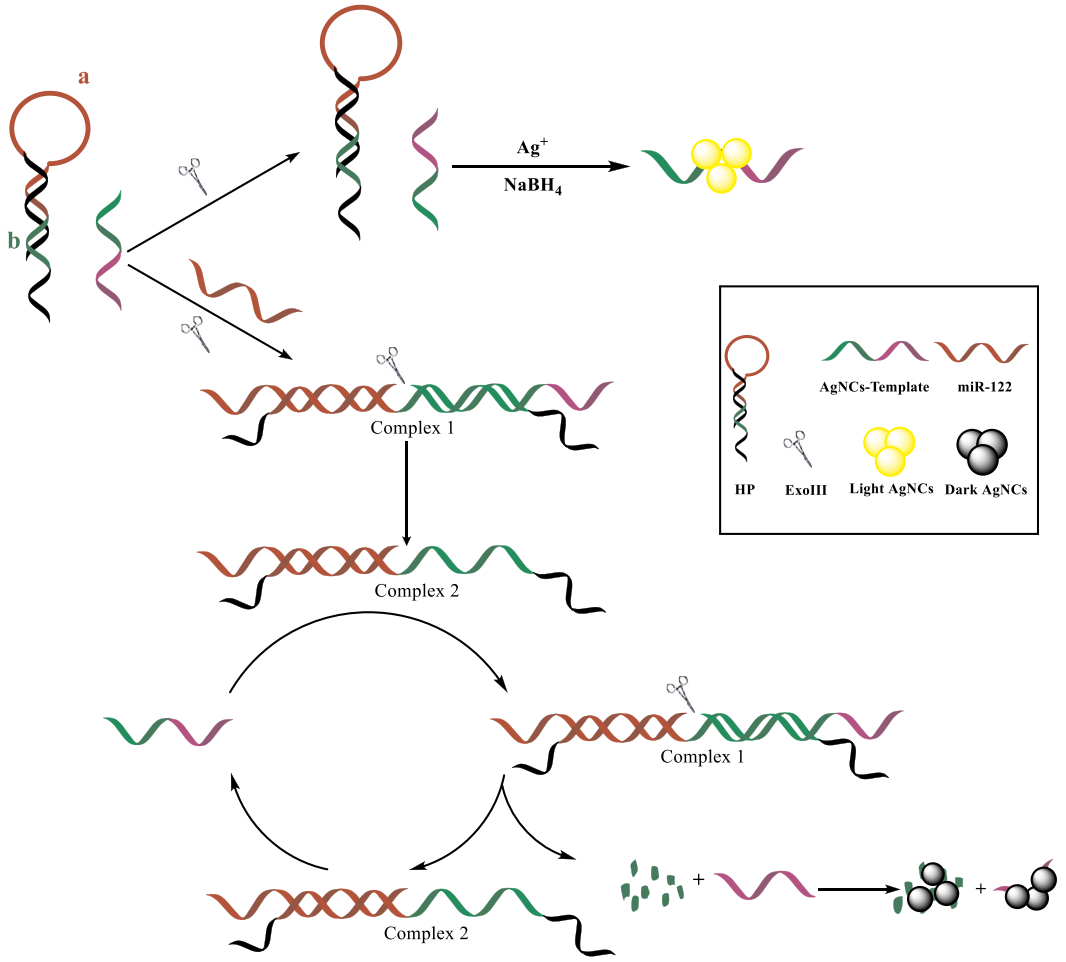

Scheme 1. Schematic illustration of the strategy based on Exo III assisted fluorescence quenching of DNA-templated silver nanoclusters to detect miR-122.

In this article, we presented a simple, rapid and labeled-free biosensor based on Exo III-assisted fluorescence quenching of DNA-templated silver nanoclusters for miR-122 in homogeneous solution [42.43]. When the target is not present, the silver cluster template cannot hybridize with hairpin probe H and cannot be hydrolyzed by Exo III, at this time, the silver cluster template can synthesize DNA-AgNCs after the addition of $AgNO_3$/$NaBH_4$, showing a strong background signal. When the target is present, it opens hairpin H to initiate the next enzymatic cleavage reaction and cyclic reaction, resulting in the hydrolysis of the silver cluster template and fail to form DNA-AgNCs, and the fluorescence intensity of the system decreases. By comparing the changes of fluorescence signals of the system before and after target addition, the high sensitivity and rapid analysis of the target can be achieved within 45 min.

2. Experimental section

2.1. Reagents and materials

Diethypyrocarbonate (DEPC)-treated water, Trihydroxymethyl aminomethane (Tris), RNase-free centrifuge tubes and tips, HPLC purified DNA and miRNA sequences were purchased from the Sangon Biotechnology Co. Ltd. (Shanghai, China). All DNA and RNA sequences are listed in Table S1. Exo III and Agarose G-10 were purchased from New England Biolabs (USA) and Sigma (Spain), respectively. The sodium nitrate ($NaNO_3$) and magnesium nitrate $Mg(NO_3)_2{\cdot}6H_2O$ used in this experiment were purchased from Xilong Chemical (Guangdong, China); sodium acetate (NaAc), potassium acetate (KAc), and magnesium acetate $Mg(Ac)_2$ were purchased from Huawei Ruike Chemical Co.Ltd.(Beijing, China); Sodium borohydride ($NaBH_4$), boric acid (H_3BO_3), disodium ethylenediaminetetraacetic (Na_2EDTA) were purchased from Sinopharm Chemical Reagent Co.Ltd.(Shanghai, China); Silver nitrate ($AgNO_3$) was purchased from Yinuokai Technology Co.Ltd.(Beijing, China). All reagents are of analytical grade and Laboratory water is ultrapure water (> 18.2 MΩ/cm). Human serum samples were provided by the Guilin's Fifth People's Hospital (China).

2.2. Apparatus

Fluorescence analysis was conducted by using LS-55 luminescence spectrometer (PerkinElmer, USA) with a parameter voltage of 750 V, a slit width of 10.0 nm, an excitation wavelength of 560 nm, an emission wavelength of 616 nm, a scanning range of 580–720 nm, and a scanning speed of 800 nm/min. Gel electrophoresis analysis using DYY-6C electrophoresis apparatus (Liuyi Instrument Factory, China)。

Gel electrophoresis analysis was performed with 4.5% agarose. The gel was run for 1.5 h in 1 × TBE (90 mM Tris-H_3BO_3, 2 mM Na_2EDTA , pH 8.0) at 95 V. After that, the gel was placed in EB solution for staining for 30 min, and the results of electrophoresis were recorded with the omega-16ic gel imaging system.

2.3. Analysis of the target miR-122

The DNA-AgNCs was prepared by some modification according to the method in literature [42,43]. A certain amount of hairpin H (30 nM), silver cluster template (1 μM), Exo III(6U) and miR-122 of