A fluorescence turn-on biosensor based on gold nanoclusters and aptamer for alpha fetoprotein detection

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Abstract. Alpha-fetoprotein (AFP), an important tumor marker, is widely used for the diagnosis of patients with germ cell tumors and hepatocellular carcinoma. Herein, a highly sensitive aptasensor for AFP detection was constructed based on fluorescence resonance energy transfer (FRET) between 5-carboxyfluorescein (FAM) and gold nanoclusters (AuNCs). AuNCs can effectively quench the fluorescence of FAM-AFP aptamer via strong coordination interactions. When AFP was introduced into the FAM-AFP aptamer-AuNCs FRET system, the AFP aptamer preferentially combined with AFP accompanied by conformational change, which greatly weakened the coordination interaction between the AFP aptamer and AuNCs. Thus, fluorescence recovery of FAM was observed. In the range of 10.0 -100.0 ng/mL, a linear relationship between the fluorescence recovery rate and the concentration of AFP was obtained with the detection limit of 6.631ng/mL. The highly sensitive FRET aptasensor with simple configuration showed promising prospect in detecting a variety of biomarkers.

1. Introduction

Alpha fetoprotein (AFP), an important indication of liver cancer, is a glycoprotein found in the yolk sac and secreted from the fetal liver in early embryonic life, which is later mainly derived from the liver and normally undetectable in healthy adults [1, 2]. In the serum of healthy human, the average level of AFP is below 25 ng/mL, but it increases obviously to 400 ng/mL in nearly 75% liver cancer patients [3]. Moreover, the concentration of AFP in the human body is related to the stage of disease. Therefore, the reliable analytical methods to identify AFP is of great significance for the early clinical diagnosis and the long-term treatment [4].

Recently, gold nanoclusters (AuNCs)-based fluorescent assay have been rapidly developed possessing simplicity in approach, shortened response time, and cost-effectiveness [5]. AuNCs show discrete electronic states due to strong quantum confinement effects, and exhibit molecule-like properties in the absorption and fluorescence features [6]. In most cases, AuNCs are adopted as the part for the “off” signaling because AuNCs could interact with the target and quench the fluorescence [7].

In the present study, a simple, highly sensitive AuNCs-based fluorescence resonance energy transfer (FRET) aptasensor for AFP detection is presented. In the introduced aptasensor, 5-carboxyfluorescein (FAM) modified AFP aptamer (FAM-AFP aptamer) was adsorbed on AuNCs via strong coordination interactions between oxygen or nitrogen atom of the nucleobase in the AFP aptamer and the Au atom of the AuNCs. Therefore, AuNCs quench the fluorescence emission intensity of the FAM. But in the presence of AFP, the AFP aptamer preferentially combined with AFP accompanied by conformational change, which greatly weakened the coordination interaction between
the AFP aptamer and AuNCs, thereby the fluorescence recovery of FAM was observed in a AFP concentration-dependent manner.

2. Experimental

2.1 Reagents and apparatus
All reagents were analytical grade and obtained from commercially available suppliers (Shanghai Linc-Bio Science Reagent and Sigma-Aldrich) and used without further purification. An ssDNA aptamer for AFP was employed as the targeting ligand [8]. The sequence of the FAM-AFP aptamer was 5′-FAM-GTGACGCTCTAAACGCTGACTGAGTTGCACTGCACTGATGATGGTGGTCTGGTCGTCCGAAACCAATC-3′. Aptamer with the complete sequence was synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). All solutions were prepared with ultrapure water of 18 MΩ·cm purified from Milli-Q purification system (Milli-Pore, Bedford, MA, USA).

Fluorescence measurements were performed on an F-4600 fluorometer (Hitachi Co. Ltd., Japan).

2.2 Preparation of the AuNCs
Gold nanoclusters were synthesized with bovine serum albumin as both the reducing agent and the template material [7]. In brief, 25 mL 10 mM HAuCl₄ was well mixed with 25 mL 50 mg/mL BSA solution, and 3 mL 1 M NaOH solution was added and then incubated on a 37 °C water bath for 8 h with continuous stirring. The obtained mixture was stored in 4 °C in dark before use.

Through the simple mixing and an incubation process, AuNCs with diameter around 6.0 nm were obtained. These AuNCs exhibited strong fluorescence emission at λex/λem 480/520 nm and appeared reddish brown color in natural light.

2.3. Construction of the FRET aptasensor and detection of AFP
To construct the FRET aptasensor, 20μL AuNCs (0.05 mg/mL) and 20μL FAM-AFP aptamer (140 nmol/L) were added to 200 μL HEPES buffer solution (20 mM, pH 6.0, containing 5 mM KCl and 5 mM MgCl₂) and the resulting solution was incubated for 60 min and the fluorescence intensity was measured.

In the present work, the FAM-AFP aptamer- AuNCs ensemble assay in HEPES buffer solution was employed for the determination of AFP. This aptasensor exhibited a very weak fluorescence emission in the absence of AFP (F₀). The fluorescence intensity of the aptasensor was recovered upon addition of various concentrations of AFP (F₁). The maximum fluorescence intensity was obtained in 520 nm after 70 min under excitation at 480 nm.

3. Results and discussion

3.1 Principle of the fluorescence turn-on biosensor for AFP detection based on FAM-aptamer and AuNCs
The AFP aptasensor was constructed based on aptamer-bridged FRET between FAM and AuNCs, as shown in Figure 1. In our design, FAM-AFP aptamer was adsorbed on AuNCs via strong coordination interactions between oxygen or nitrogen atom of the nucleobase in the AFP aptamer and the Au atom of the AuNCs [9]. The strong coordination interactions brought the fluorescence donor FAM close to the fluorescence acceptor AuNCs, which resulted in the occurrence of FRET, and the fluorescence quenching of FAM was observed. After AFP was introduced into the FAM-AFP aptamer-AuNCs FRET system, AFP aptamer preferentially bound to AFP accompanied with its conformational change, which largely weakened the coordination effect between the AFP aptamer and AuNCs. Thus FAM was separated from AuNCs and the FRET process was blocked. Meanwhile, the fluorescence recovery of FAM was observed and the degree of fluorescence recovery was in a positive AFP concentration-dependent manner.
3.2 The feasibility of fluorescence AFP detection based on FAM-aptamer and AuNCs

The feasibility of fluorescence AFP detection based on FAM-aptamer and AuNCs was evaluated at different systems with PBS solutions. From Figure 2A, in the absence of AFP, the fluorescence intensity of the system (red line in Figure 2A) was lower. This was that the fluorescence intensity of FAM-aptamer was quenched through FRET process (FAM-aptamer as donor and AuNCs as acceptor) due to FAM-aptamer would absorb onto the AuNCs surface via coordination interactions. While in the presence of AFP (blue line in Figure 2A), the fluorescence of the system recovered to high intensity. In addition, the fluorescence of the system recovered better with the increasing concentrations of AFP (black line in Figure 2A). This was the fact that AFP with high affinity bounded FAM-aptamer and could not be absorbed onto AuNCs, which could block the FRET process and consequently decrease the fluorescence quenching. The degree of fluorescence recovery was in a positive AFP concentration-dependent manner. This phenomenon explained that the present aptasensor was constructed based on the principle of FRET between FAM-aptamer and AuNCs.

3.3 Construction of the FRET aptasensor

To establish a FRET assay with high quenching efficiency, it is important to optimize the ratio between the donor molecules and the quencher molecules. In order to get the optimal ratio between FAM-labeled AFP aptamer and AuNCs for maximum quenching efficiency, 20 μL FAM-AFP aptamer (140 ng/mL) and 20 μL of a series of concentrations of AuNCs ranging from 0.01 mg/mL to 0.10...
mg/mL were added into 200 μL HEPES buffer solution and incubated for 60 min and measured the fluorescence signals. Figure 2B showed the fluorescence spectra of FAM-aptamer in the presence of different concentrations of AuNCs. It can be seen from Figure 2B that AuNCs can interact with FAM-AFP aptamer and quench its fluorescence. The concentration of 0.05 mg/mL for AuNCs was the optimum quenching effect to the FRET aptasensor.

3.4 Quantitative determination of AFP with the FRET aptasensor

Under the optimized experimental conditions, the AFP detection was performed by the FRET aptasensor. The fluorescence recovery rate \( (F_1-F_0)/F_0 \) was a AFP concentration-dependent manner, as indicated in Figure 3A. A linear relationship between the fluorescence recovery rate of FAM and the concentration of AFP in the range of 10.0-100.0 ng/mL was obtained (Figure 3B). The regression equation was \( Y=0.00133X+2.508 \) with a correlation coefficient of 0.99661. The detection limit for AFP, based on three times of the signal-to-noise level, was calculated to be 6.631 ng/mL.

Figure 3 (A) The fluorescence recovery trend line in accordance with different concentration of AFP. (B) The linear relationship between the fluorescence recovery degree and the concentration of AFP within the range of 10.0-100.0 ng/mL.

4. Conclusions

In summary, a highly sensitive FRET aptasensor for AFP detection has been constructed based the excellent fluorescence quenching ability of AuNCs towards FAM. The application of AFP aptamer with high affinity and specificity towards AFP also contributed to the good performance of this biosensor. In the range of 10.0-100.0 ng/mL, a linear relationship between the fluorescence recovery rate of FAM and the concentration of AFP was obtained with a readily achievable detection limit of 6.631 ng/mL. This homogeneous FRET aptasensor offers great application promises for detection of plasma AFP.

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