Effects of Different Buffers on the Construction of Aptamer Sensors

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Abstract. In this paper, the effect of different buffers, PBS and TBE, on the construction of an aptamer sensor (apt sensor) for ATP was investigated. The apt sensor was based on fluorescence energy resonance transfer (FRET), when the energy donor was 5'-carboxyfluorescein (5'-FAM) and the energy receptor was Au nanoparticles (AuNPs), respectively. Both the donor and acceptor were conjugated with complementary and single stranded DNA (ssDNA). The fluorescent changes of the sensors were measured to investigate the influence of different buffers during the whole preparation and detection process. The results indicated that when the AuNPs and ssDNA (Au-DNA1) were conjugated in PBS buffer, the corresponding apt sensors would obtain a better detection ability of ATP than in TBE buffer.

1. Introduction
Nowadays, aptamers, single-stranded nucleic acids, get a great improvement. They can be artificially selected from DNA libraries by Systematic exponential concentration of ligand evolution (SELEX), chemically synthesized, as well as have excellent selectivity and special binding to many targets, even small molecules [3]. Therefore, the aptamer based biosensor (apt sensor) became a promising research field [1-2], such as electrochemical sensors [4], chemiluminescence sensors [3], fluorescent sensors [5], etc. Among them, the fluorescent biosensors based on fluorescence resonance energy transfer (FRET) have high sensitivities, specificities and simply detection methods [5].

ATP is the main source of energy required for tissue cells in the body, and the synthesis of important substances in the body requires ATP participation [4-5]. The apt sensors for ATP have a great improvement in different fields, such as cell imaging [6]. According to FRET mechanism, the energy donor and acceptor of an apt sensor should be conjugated to two complementary single-stranded DNA (ssDNA), respectively. Therefore, the preparation of fluorescent apt sensors is similar to the traditional method of fluorescent biosensors for DNA. In previous researches, different buffers, such as PBS and TBE, were used in the preparation of apt sensors. Zhao et al. prepared a DNA biosensor with good stabilities in PBS buffer [6]. Wu et al. investigated the conjugations of DNA and Au nanoparticles (AuNPs), it was found that TBE buffer could make the system of AuNPs better stability, and Tris-HCl buffer could make DNA connection rate lower [7]. Therefore, the stability of construction of the sensor has become a focus of biosensors, and different buffers have different effects on the construction of the sensor [8]. In this work, a FRET-based apt sensor is presented to
investigate the effect of different buffers on the process of sensor preparation for the further establishment of higher sensitivity and specificity selection conditions for ATP aptasensors.

2. Experiment

2.1. Reagents and instruments
HAuCl₄⋅4H₂O were from Kernel Chemical Reagent Company (Tianjin, China). Tris(hydroxymethyl)-aminomethane was obtained from Alfa Aesar. ATP was purchased from Sigma-Aldrich. All other reagents were of analytical grade. The oligonucleotides, ATP aptamer (DNA₁), and its complementary strand (DNA₂) were supplied by the SBS Genetech Company (Beijing, China). The base sequence of the DNA₁ was 3′-SH-(CH₂)₆-TGG AAG GAG GCG TTA TGA GGG GGT CCA-5′, and the sequence of DNA₂ was 3′- AACGC CTC CTT CCA-FAM-5′.

2.2. Preparation of AuNPs
The Fren’s method was utilized to obtain AuNPs of 16nm in diameters when the citrate was as the reduction [4]. HAuCl₄ (1mL, 1%) was added into 100mL of water and fluxed till boiling. Then, trisodium citrate solution (2.5mL, 1 %) was quickly added into this mixture with strong stirring for 15 min. After the color of reaction system became wine red, the reaction should be stopped and cooled to room temperature. The AuNPs were separated and purified by centrifugation (10,000 r/min about 40 min), resuspended in aqueous solution (100mL), and stored at 4° C. The UV-vis absorption spectra and transmission electron microscopy (TEM) of AuNPs were measured by Helios-γ thermos ultraviolet and visible spectrophotometers (USA) and JOEL JEM-2100F Fas TEM (Japan), respectively. The concentration of the AuNPs solution is estimated by the following method. The concentration of AuNPs is 2.3nmol/L [9].

2.3. The construction of Au-DNA conjugations (Au-DNA₁)
DNA₁ and AuNPs were incubated at room temperature for about 16h when 3′-SH-DNA (DNA₁, 1OD) was mixed with AuNPs suspension (7mL). Then, the solution was transferred into a 10mmol/L PBS buffer with 0.1mol/L of NaCl (pH=7.0) under shake at 37 °C. After 40 h, the reaction could be ended, and DNA₁ conjugated AuNPs (Au-DNA₁) obtained. The resultant Au-DNA₁ conjugates were purified by ultracentrifugation of 10,000r/min and resuspended in PBS.

2.4. Preparation of FRET-based aptasensors
The apt sensor was obtained after the hybridization of Au-DNA₁, and DNA₂ was incubated in a Tris-HCl buffer at 37°C for about 12h. The detection process was that an amount of ATP was introduced into the apt sensor detection solution under shake. After 12h, the fluorescent spectra of the detecting solution were measured by F-7000 fluorescence spectrophotometer (HITACHI, Japan). The quenching efficiency (Q%) was given as follows:

\[ Q\% = \frac{F-F_0}{F} \]  

(1)

3. Results and Discussion

3.1. The effect of the buffers in the AuNPs and Au-DNA₁
The ratio of AuNPs suspension and PBS buffer solution for the conjugation of AuNPs and ssDNA (DNA₃) was controlled at 1:4 (v/v), which was the best ratio according to the reference work [10]. The investigation of the bioconjugations between AuNPs and DNA₁ in PBS buffer by centrifugation at 10,000 r/min was shown Figure 1, when the mole ratio of AuNPs to DNA₁ was 1:200 and the DNA₁ concentration was 10mM.
Figure 1. (A) TEM of AuNPs. (B) UV-vis of Au-DNA1 suspension system. (a) precipitation after centrifugation; (b) The supernatant after centrifugation; (c) The supernatant after second centrifugation.

The AuNPs were uniform in size (about 16nm), good dispersion without agglomeration (Figure 1. A). After centrifugation, the Au-DNA1 conjugations were separated from the suspension system and showed a strongly characteristic absorption peak at 520 nm of AuNPs as well as a weakly characteristic absorption peak at 260 nm of DNA (Figure 1. B (a)), while the supernatant after centrifugation only had a characteristic absorption peak at 260 nm of DNA (Figure 1. B (b)), which indicated that there were no AuNPs in supernatant. Then, when the precipitation of Au-DNA1 conjugations was re-suspended into PBS buffer solution and re-centrifuged, the supernatant had no DNA absorption at 260 nm, which indicated that the DNA1 were conjugated with AuNPs [12], successfully (Figure 1. B (c)).

Figure 2. UV-vis of Au-DNA1 precipitations in (A) PBS and (B) TBE buffer. The molar ratio of Au:DNA1 was (a) 1:50; (b) 1:100; (c) 1:150; (d) 1:200; (e) 1:225.

After that, the molar ratios of AuNPs to DNA1 were explored in PBS and TBE buffer, respectively (Figure 2). With the increase of the amount of DNA1, the absorption peak of Au-DNA1 precipitations at 260 nm of DNA1 was increased, which indicated that AuNPs carried more DNA1. Moreover, the absorption peak of Au-DNA1 precipitations at 520 nm of AuNPs was also increased, which indicated an increase of stability and dispersity of Au-DNA1 at high DNA concentration because of the hydrophilicity of DNA1[9]. In the PBS buffer solution, when ratio of Au: DNA1 was from 1:50 to 1:150, the absorption peaks of Au-DNA1 conjugations had a steady rise. When the ratio of Au: DNA1 was 1:200, the Au-DNA1 absorption peak had a significant increase. However, when the ratio Au: DNA1 was increased to 1:225, the absorption peaks had no obvious changes, which indicated that the conjugation reaction between AuNPs and DNA1 was reached the equilibrium. However, in TBE buffer, the absorption peaks of Au: DNA1 had a steady increase following the increase of DNA1 amount, which indicated that the conjugation reaction between AuNPs and DNA1 was not reached equilibrium.
Therefore, the AuNPs could be coated with more DNA$_1$ which resulted in a better dispersity of Au-DNA$_1$ at the same Au: DNA$_1$ ratio in PBS buffer.

3.2. Construction of fluorescence resonance energy transfer system
The necessary condition for the FRET is that the UV absorption spectrum of the energy donor overlaps with the fluorescence spectrum of the energy receptor [13]. In this work, the fluorescence emission peak of 5'-FAM of DNA$_2$ was at 519 nm, and the UV absorption peak of AuNPs was 520 nm, which was consistent with the FRET theory. In above part, the acceptors, AuNPs, were conjugated with DNA$_1$. When the DNA$_2$ existed, the apatsensors could be obtained by the hybridization of DNA$_1$ and DNA$_2$ in Tris-HCl buffer. The fluorescent results and corresponding quenching efficiency (Q%) of apatsensors prepared by Au-DNA$_1$ in PBS and TBE buffer at different Au: DNA$_1$ ratio (1:50, 1:100 and 1:200) were shown in Figure 3. The Tris-HCl was as hybridization buffer, because it could provide a better objective condition, so that the base pairing of double-stranded DNA (dsDNA) could be more stable and reduces the insertion of erroneous bases [12].

![Figure 3](image.png)

**Figure 3.** fluorescent intensities of apt asensors prepared by Au-DNA1 in (A)PBS; (B)TBE buffer; (C) corresponding quenching efficiency at different Au: DNA1 ratio. (a) no Au-DNA1; (b) 1:50; (c) 1:100; (d) 1:200.

The increase of DNA$_1$ could result in the increase of Q% (Figure 3A and B). Moreover, the Q% of fluorescent apt asensors prepared by Au-DNA$_1$ in PBS had a significantly increase than sensors prepared in TBE (Figure 3C), which also confirmed that the Au-DNA$_1$ prepared in PBS buffer could carry more DNA$_1$.

3.3. Detection of the fluorescent apt asensors for ATP

![Figure 4](image.png)

**Figure 4.** Fluorescence detection of apt asensors prepared by Au-DNA1 in (A)PBS; (B) TBE buffer, with (a) only DNA2; (b) apt asensors without ATP; (b) apt asensors with ATP. When the mole ratio of Au: DNA1: DNA2 was 1:200:200 and the ATP concentration was about 1mM.

Because the ATP molecule is complementary with the DNA$_1$, the hybridized structure of dsDNA in apt asensors will be opened when the ATP exists, which will result in the fluorescent recovery of apt asensors. The detection of apt asensors was investigated when the ratio Au: DNA$_1$ was 1:200, because
the conjugation reaction between AuNPs and DNA$_1$ reached the equilibrium at this ratio in PBS buffer and the detection were shown in Figure 4. The results indicated that when ATP exists (1mM), the increase of fluorescent intensity was about 52.51% when the apt sensors were prepared by Au-DNA$_1$ in PBS buffer, while the increase of fluorescent intensity was about 33.60 % when the apt asensors were prepared by Au-DNA$_1$ in TBE buffer. Therefore, the apt sensors prepared by Au-DNA$_1$ in PBS buffer should had a better detection ability.

4. Conclusion

Two kinds of buffers were explored in the construction of an apt sensor for ATP based on FRET theory. The PBS buffer showed better characteristics. The connection of AuNPs and DNA$_1$ in PBS is more stable than in TBE buffer. Moreover, the apt asensors prepared by Au-DNA$_1$ in PBS buffer showed a better fluorescent quenching and recovery abilities. This conclusion provided a basis for the future application of apt sensors and specific testing to supply a good buffer environment.

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