

Enzyme Functionalized AuNPs and Glucometer-based Protein Detection

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Abstract: We here developed a novel method for protein detection by using protein aptamer-functionalized magnetic beads for protein recognition and invertase -functionalized AuNPs catalyze sucrose generate glucose that can be detected by a glucometer. First, the invertase and DNA probe P2 are immobilized onto the gold nanoparticles (I/P2@AuNPs). Next protein aptamer P1 are immobilized onto the streptavidin-coated Magnetic beads (P1@MB). P1 and P2 can complementary to form double-stranded DNA. When target protein presence, P1 combine with target and release I/P2@AuNPs. Then magnetic separation, take supernatant fluid and add sucrose after a period of reaction, detection of glucose concentration by glucometer, thus achieve the sensitive and selective detection of the target protein.

1. Introduction

To our knowledge proteins play critical roles in a variety of life activities [1]. So highly sensitivity and selectivity protein detection methods are particularly important in clinical medicine, disease diagnosis [2], biology and food safety [3]. Over the past decades, the detection of different types of protein has attracted many researchers' interests. In order to improve the sensitivity and selectivity of the methods, a mass of biomaterials, such as gold nanoparticles [4], silver nanoparticles [5] have been used to the detection of protein. They have favourable biocompatibility [6] and can be easily connected to the biological macromolecules. In addition, the functionalized magnetic beads (MBs) were also applied for the protein detection, because of the DNA aptamers or antibodies can be easily immobilization onto the surface of MBs with the effect of chemical coupling or physical adsorption [7]. Meanwhile the separation of magnetic beads are very convenient and effective, This is good for the following study.

Recently, various signal amplification strategies based on different enzymes [8], sucrase is a kind of common enzyme. Not only sucrase has a strong catalytic ability but sucrase catalytic sucrose generate glucose can be combined with our common testing instrument - glucometer. With the help of these reagents and instrument, we constructed a sensitive, selective and convenient method for detection of protein.

2. Materials and Methods

2.1. Materials and Reagents

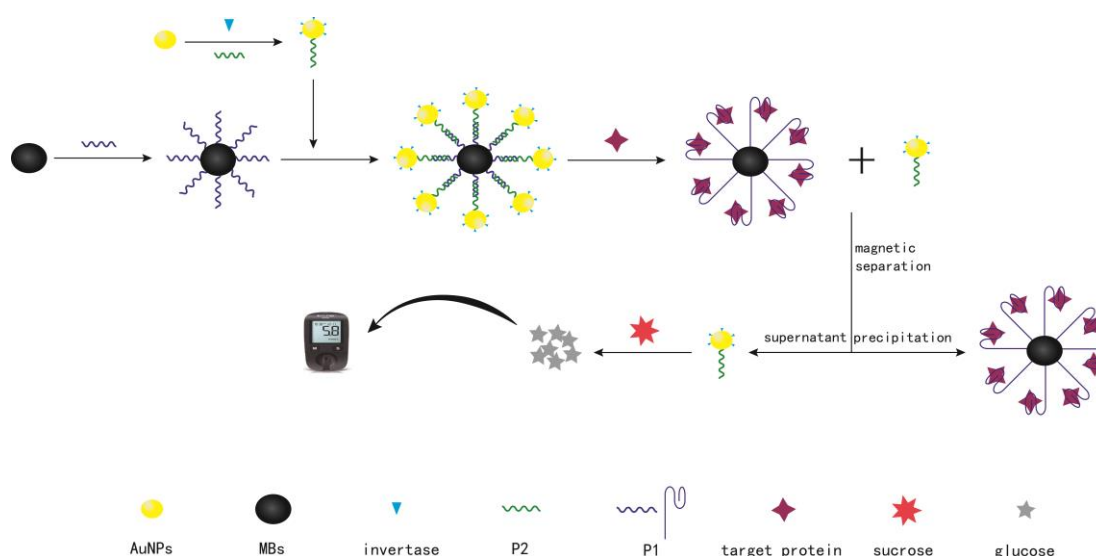
Streptavidin-coated magnetic beads (1 μ m in average diameter) were purchased from NEW ENGLAND BioLabs Inc. All oligonucleotide sequences and 1 \times PBS were purchased from Sangon Biotechnology. Co, Ltd (Shanghai, China). Grade VII invertase from baker's yeast (*S. cerevisiae*), and



gold acid chloride trihydrate, were the products of Sigma-Aldrich. Ultrapure water (Milli-Q 18.2 M, Millipore System Inc.) was employed in all runs. All other chemicals were of reagent grade and used as received.

2.2. Methods

This study aimed at the development of sensitive and selective method for the quantitative detection of proteins. A schematic of this proposed concept is presented in **Scheme 1**. First the streptavidin-coated magnetic beads were conjugated with biotin-labeled DNA probe P1 (P1@MBs), P2 and invertase were immobilization on the AuNPs (I.P2@AuNPs). The P1@Mbs and I.P2@AuNPs form I.P2 P1@MBs. When the target protein presence the aptamer (P1) will combine with the targets and release I.P2@AuNPs. Then magnetic separation, take the supernatant and add sucrose solution after reaction a period of time, use glucometer (ACCU-CHEK Active) measure the concentration of glucose.



Scheme 1. Illustrate the process of experiment

2.2.1 Preparation of I.P2@AuNPs. Gold nanoparticles (26 nm) were prepared as previous work [9,10]. Briefly, 10 μ L of HAuCl₄ solution was added into 96 mL of ultrapure water, and the mixture was boiled with vigorous stirring. Then, 4 mL of trisodium citrate solution (1%, m/v) was quickly injected into the boiling solution. The solution was kept stirring and heating to its color changed from pale yellow to wine red. After stopt stirring and cooled to room temperature, the resulting solution was stored in brown glass bottles at 4 °C for future use.

As shown in **scheme 1** The I.P2@AuNPs were synthesized with AuNPs, P2 and invertase according to the literature [11,12,13]. First, invertase (80 μ L, 30 mg/mL) was added into 400 μ L of the AuNPs solution. The resulting mixture was incubated for 20 min at room temperature with gentle shaking. During this process, invertase were covalently bound to AuNPs via the dative binding between AuNPs and free -SH groups of the invertase. Then, DNA probe P2 (20 μ L, 1.0 μ M) dissolved into 10 mM Tris-HCl buffer including 10 mM TCEP and 0.1 M NaCl (pH 7.4) was added to the mixture, and incubated overnight. Finally, the resulting mixture was centrifuged (13,400g) for 20 min at 4 °C and stored at 4 °C (400 μ L \times PBS, pH 7.4) until use.

2.2.2 Preparation of I.P2 P1@MBs. A portion of 0.96 mL 1 mg/mL solution of streptavidin-coated magnetic beads (MBs) was placed close to a magnetic rack for 1 minute. The clear solution was discarded and replaced by 0.96 mL of PBS. This buffer exchange procedure was repeated twice. Then, 32 μ L of 100 μ M biotin-DNA (P1) was added to the MB solution and well mixed for 30 minutes at room temperature. After that, the MBs were washed twice using buffer to remove excess P1.

Dissolved the precipitation get P1@MBs solution. Later, 32 μL of 100 μM I.P2@AuNPs were added to the P1@MBs solution and well mixed for 30 minutes at room temperature. Using magnetic separation and 1 \times PBS buffer washing three times to remove excess I.P2@AuNPs. then the as-prepared I.P2 P1@MBs were dispersed in 1 \times PBS.

First the streptavidin-coated magnetic beads were conjugated with biotin labeled DNA probe P1 according to the protocol suggested by the manufacturer. Next, magnetic separation and removed the supernatant, resuspension the precipitate in 1 \times PBS and get P1@MBs solution. Then, excess I.P2@AuNPs are added to the P1@MBs solution, after an hour of reaction, the excess I.P2@AuNPs were washed three times by 1 \times PBS buffer. The I.P2 P1@MBs were dispersed in 1 \times PBS until use. All the processes shown in **scheme 1**.

3. Results

First we characterization of gold nanoparticles by transmission electron microscopy (Hitachi-7500, Japan) and the result shown in **figure 1**. It reveals that the synthesis of AuNPs have a more uniform diameter size. Then we optimized the invertase concentration of synthesis of invertase@AuNPs. The concentration of invertase from 30 mg/mL to 70 mg/mL was used in the experiment. Next characterization of the invertase@AuNPs by UV-Vis spectra (Thermo Nanodrop-1000, USA) shown in **figure 2**. In the subsequent experiments We choose the concentration of invertase as 30 mg/mL.

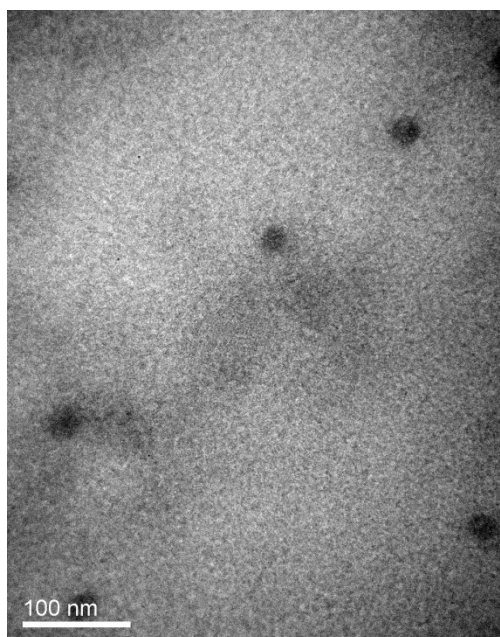


Figure 1. The transmission electron microscopy map of gold nanoparticles.

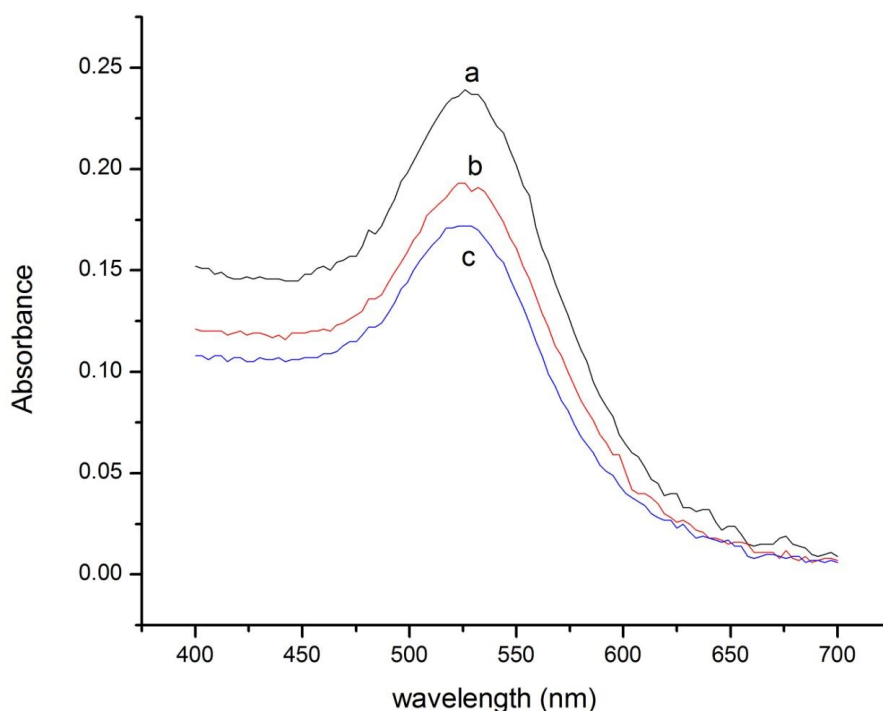


Figure 2. UV-Vis spectra of invertase@AuNPs .The invertase concentration of synthesis of invertase@AuNPs: (a) 30 mg/mL, (b) 50 mg/mL, (c) 70 mg/mL.

4. Conclusions

In this study I.P2 P1@MBs were prepared via streptavidin-coated magnetic beads and invertase labelled AuNPs through P1 and P2 hybridization. Furthermore, we optimized the invertase concentration of synthesis of invertase@AuNPs and utilized UV-Vis spectra characterization to get the best concentration 30 mg/mL. What we composited are great potential to detect proteins.

5. References

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