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A nanoresonant gold-aptamer probe for rapid and sensitive detection of thrombin

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Abstract Resonance light scattering (RLS) is a sensitive technique for monitoring scattered light induced by extended aggregates of chromophores. It has been widely used to study aggregations for its simple manipulation, high sensitivity and great versatility. Gold nanoparticles generate colorful light-scattering signals due to their unique surface plasmon resonances, hence extraordinary light scattering upon aggregation. In this paper we report a rapid and sensitive method based on gold nanoparticles and DNA aptamer to detect protein biomarkers by RLS. Thiol modified thrombin aptamer was covalently assembled to the surface of gold nanoparticles as nanobio probes. As thrombin has two specific binding sites for its aptamer, it can bridge the well dispersed nanoparticles and lead to a network of particle aggregations. The formation of aggregation ia measured by RLS, and the specific detection of thrombin at nM level is achieved. The method has good specificity.

Key words Resonance light scattering, Nanoprobes, DNA aptamer, Protein detection

1 Introduction

Quantitative detection of protein becomes increasingly important in fundamental research and clinical practice. Immunological assays based on the use of antibodies are widely used for protein detection, but they suffer from the limitations of sensitivity, selectivity and stability. Aptamers are RNA or DNA oligonucleotides that interact with their specific targets with high affinity and specificity[1,2]. They possess the properties of antibodies but have higher sensitivity, selectivity and stability, thereby are generally considered as substitutes for antibodies. And aptamer, with its great flexibility, has been used for several years for developing protein assays based on electrochemical, fluorescence, surface plasmon resonance or quartz crystal microbalance, etc[3-10].

Resonance light scattering (RLS) is a sensitive technique for monitoring light scattered from the extended aggregates of chromophores[11], which has been widely used in the detection of DNA, proteins and small molecules, owing to the simple manipulation, high sensitivity and great versatility[12-15]. However, organic dye-based RLS protein assays are of relatively poor sensitivity[16,17]. It was found that gold nanoparticles (AuNPs) scatter lights extraordinarily at the plasmon-resonance wavelength due to the collective oscillation of their conduction electrons upon aggregation, thereby this may provide a way to replace organic dye as promising RLS tags[11, 18-20].

In this paper, Au nanoparticles are used to develop an RLS-based thrombin assay of high sensitivity and selectivity. And the thrombin-specific aptamer is employed as an example to show the importance of RLS technique in protein detection.

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2 Experimental

As shown in Fig.1, thiol modified thrombin-specific aptamer was covalently modified on the surface of gold nanoparticles as nanoprobes. Thrombin has two specific binding sites for its aptamer[21], upon the addition of thrombin, the well dispersed nanoprobes were bridged together and formed a network of particle aggregations. By examining the formation of aggregation by RLS, rapid and sensitive detection of thrombin can be realized.

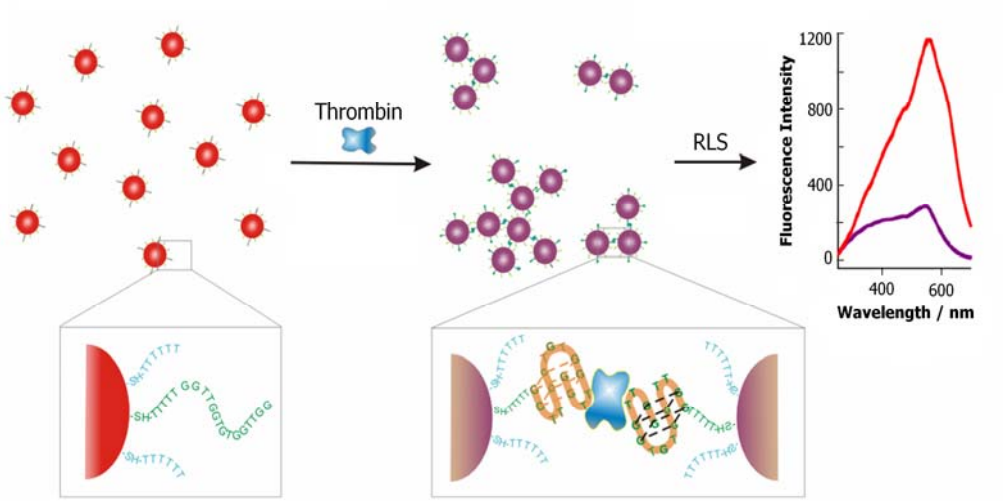

Fig.1 Illustration of the detection strategy for thrombin using nanoprobes and resonance light scattering technique.

2.1 Materials and instrument

Hydrogen tetrachloroaurate (III) ($HAuCl_4.4H_2O$, 99.99%) was purchased from China National Pharmaceutical Group Corporation. Thrombin and bovine serum albumin (BSA) were obtained from Sigma-Aldrich. All other chemicals were of analytical grade. All chemicals were used without further purification. Water was purified using a Millipore filtration system. Gold nanoparticles (AuNPs) of 13 nm size were synthesized by citrate reduction of $HAuCl_4$ as previously reported[22]. DNA sequence1: 5'-SH-TTTTTGGTTGGTGTGGTTGG-3' and DNA sequence 2: 5'-SH-TTTTTT-3' were synthesized by Shengong Co. Ltd. (Shanghai). RLS signal was measured with Hitachi F4500 Fluorospectrometer.

2.2 Preparation of nanoprobes

The preparation of nanoprobes is based on the protocols developed in our laboratory[23-26]. In brief, the thrombin- specific aptamer (DNA sequence 1) and a co-assembly strand (DNA sequence 2) were added to an aqueous solution of AuNPs (10 nM) to a final probes concentration of 3 μmol·L^{-1} and 2 μmol·L^{-1}, respectively. After incubation for one hour, 1M PBS solution (100 mmol·L^{-1} phosphate, 1 mol·L^{-1} NaCl, pH 7.4) was added to a final concentration of 0.1 mol·L^{-1}. The mixture was aged for 16 hours at room temperature. Excess oligonucleotides were removed by centrifugation at 12000 rpm for 15 min at 4ºC. The deposit was washed with 0.1M PBS for three times. Finally, the prepared nano-probes were dispersed in thrombin binding buffer (22 mmol·L^{-1} Tris-HCl, pH 7.8, 120 mmol·L^{-1} NaCl, 50 mmol·L^{-1} KCl, 0.85 mmol·L^{-1} $CaCl_2$, 6 mmol·L^{-1} $MgCl_2$ and 6.8% glycerin).

2.3 Thrombin assay

The nanoprobe solution was diluted 3-fold with thrombin-binding buffer before use. Thrombin of 2 μL was added into 48 μL of the nanoprobe solution and reacted at room temperature for 45 min. In order to avoid the absorption of the thrombin on the wall of plastic tubes, so all the tubes for the thrombin reaction were blocked with 1% BSA in advance. The pre-treatment avoided the reduction of sensitivity with the loss of thrombin molecules. After the binding, the solution was diluted with 550 μL thrombin-binding

buffer. The RLS signal was collected with a Hitachi F4500 Fluorospectrometer in synchronous mode.

3 Results and discussion

Upon interaction with thrombin, the thrombin-specific aptamer underwent a conformational change from random coil to Q-quadruplex, which required sufficient space. In consideration of the crowded confined space on the surface of AuNPs, a spacer (TTTTT) sequence between the 15 bp thrombin aptamer and thiol group was designed. The co-assembled strand (DNA sequence 2) to control the density of aptamer probes on each AuNPs surface was designed, too. By measuring the reaction time, we found that the binding process could be finished in 45 min, so as to achieve stable generation of the RLS signals.

Due to their unique surface plasma resonance properties, AuNPs have been used as optical probes in chemical and biological sensors. According to RLS theory, when strong electronic coupling exists among the chromospheres, the RLS effect can be observed at the wavelength close to the light absorption of the aggregated species[11,19]. In this paper, AuNPs exhibit a characteristic plasmon absorption band around 520 nm. As shown in Fig.2, a scattering peak at 550 nm near the absorption band of AuNPs was observed, which was attributed to the resonance light scattering signal of AuNPs. As the concentration of thrombin increased, the RLS signal of 550 nmol·L^{-1} also enhanced, which indicating that the thrombin bound to its aptamer induced the aggregation of AuNPs nanoprobes.

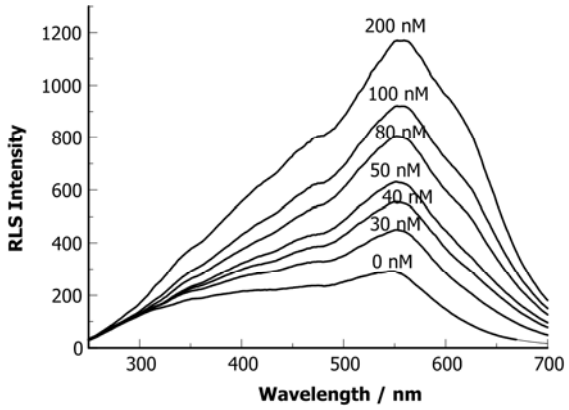

Fig.2 Resonance light scattering spectra of AuNPs nanoprobes in thrombin binding buffer of different concentrations.

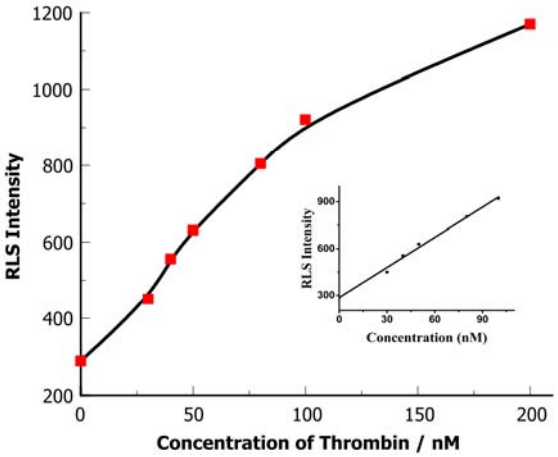

Fig.3 Dose-response curve of thrombin detection (RLS signal intensity at 550 nm versus thrombin concentration range from 0 to 200 nmol·L^{-1}). The linearly fitted calibration curve of thrombin detection (concentration range from 0−100 nmol·L^{-1}).

The relationship between thrombin concentration and RLS intensity is shown in Fig.3. The linear fitting of the calibration curve over the thrombin concentration range of 0−100 nmol·L^{-1} is shown in the insert. The limit of detection (LOD) of this assay was estimated at 30 nmol·L^{-1}. In addition, a range of non-cognate proteins (BSA, human serum albumin and human immunoglubin G) with concentration of 30 μmol·L^{-1} (10^3-fold higher than thrombin of 30 nmol·L^{-1}) led to signals indistinguishable from the background (data not shown here), which suggested good selectivity.

4 Conclusion

In summary, the present study has developed a new protein biosensors based on AuNPs, aptamers and RLS technique. The aggregation of AuNPs nanoprobes induced by the specific interaction of thrombin with its aptamers, resulting enhanced RLS signals. The results enabled the design of sensitive biosensors for other protein targets using RLS technique. Moreover, besides high sensitivity, the detection method is simple, fast and less demand of reagents.

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