Clinical Biochemistry 46 (2013) 1734–1738

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Signal enhancement in a lateral flow immunoassay based on dual gold nanoparticle conjugates

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ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 14 August 2013
Accepted 17 August 2013
Available online 28 August 2013

Keywords:
Lateral flow immunoassay
Dual gold nanoparticle conjugates
Aptamer
Signal enhancement

ABSTRACT

Objective: In order to amplify signal of lateral flow immunoassay for specific detection of thrombin.

Design and methods: A new, simple method of amplifying signals using two gold nanoparticle conjugates (GNP) in gold-nanoparticle-based lateral flow immunoassay without an additional step was developed. The first conjugates were prepared by labeling DNA1 with 30 nm GNPs, and the second conjugates were prepared by immobilizing both DNA2 and thrombin aptamer on the surfaces of 16 nm GNPs.

Results: The detection limit was improved 30 times. The lateral flow immunoassay developed in this study was applied to detect thrombin concentration in the range of 0.5–120 nM with a detection limit of 0.25 nM.

Conclusions: The lateral flow immunoassay developed in this study was used to detect thrombin concentrations within a range of 0.5–120 nM with a detection limit of 0.25 nM. This assay is very versatile and can be easily extended to other proteins.

Introduction

Recently, lateral flow immunoassay (LFIA) has attracted research interest because of its considerable advantages: user-friendly format, very short assay time (generally several minutes), little chromatographic separation interference, low cost, and no need for special training or skilled technicians [1]. This technique is based on the highly specific immunological reaction between antigens and antibodies. In the standard LIFA scheme, antibodies labeled with gold nanoparticles (GNPs) are used as affinity reagents [2]. However, any strategy based on antibodies may encounter some drawbacks because of the difficulty of antibody production, limited stability, and the complexity of modifying them. For these reasons, the identification of alternate candidates is significant. Aptamers, artificial nucleic acid ligands, are short, single-stranded oligonucleotides (RNA or DNA) that are highly specificity and have considerable affinity with target proteins [3–7]. They are also easy to synthesize, store, and label at almost any desired site without loss of activity [8,9]. For these reasons, aptamers are used to replace antibodies in protein research and biosensors [10–12]. Protein detection systems based on aptamers mostly include optical [13], quartz crystal microbalance [14], and electrochemical methods [15,16]. However, these aptamer-based analyses take a long time because of the multiple incubation and separation steps required, and this has prevented widespread application. Recently, Hui and coworkers developed a LFIA based on aptamer-functionalized gold nanoparticles, which served as probes for thrombin analysis. This process has a short assay time and low detection limit [2]. Although the aptamer-based LFIA techniques described above have lower detection limits than antibody-based LFIA, higher sensitivity is often required in clinical diagnosis, food monitoring, and chemical and biological research. The sensitivity of LFIA methods must be improved.

Gold nanoparticles are used as colored reagents in LFIA because of their vivid color and excellent chemical stability. The sensitivity of LFIA is related to the strength of the red color of the test line. Recently, a great deal of effort has been directed into improving the sensitivity of these tests. For example, a cross-flow chromatographic assay based on silver staining for improving the detection limit has been reported [17,18]. The red color of the test lines caused by gold particles primarily accumulated upon immune recognition is greatly enhanced due to the silver deposition. Unfortunately, this approach requires an additional liquid handling step for silver enhancement and thus loses the advantages of the LFIA method, specifically the possibility of a rapid one-step assay. More sensitive LFIA without an additional signal enhancing step would be extremely useful. In this study, this was achieved by using two GNP-DNA conjugates to increase the number of gold nanoparticles accumulated on the test line. The GNPs with 30 nm diameter (1st GNP) were modified with DNA1 to form a conjugate (1st GNP-DNA1). The GNPs with 30 nm diameter (2nd GNP) were modified with both DNA2 and aptamer to form the other conjugate (2nd GNP-DNA2/aptamer). The 1st GNP-DNA1 conjugate was designed to bind only with the 2nd GNP-DNA2/aptamer conjugate via hybridization between DNA1 and DNA2. The 2nd GNP-DNA2/aptamer conjugate was also able to combine with an analyte for the sandwich assay. The strategy is shown in Fig. 1. Using this strategy, a high-sensitivity one-step LFIA method for the analysis of thrombin as a model analyte was developed.

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http://dx.doi.org/10.1016/j.clinbiochem.2013.08.010

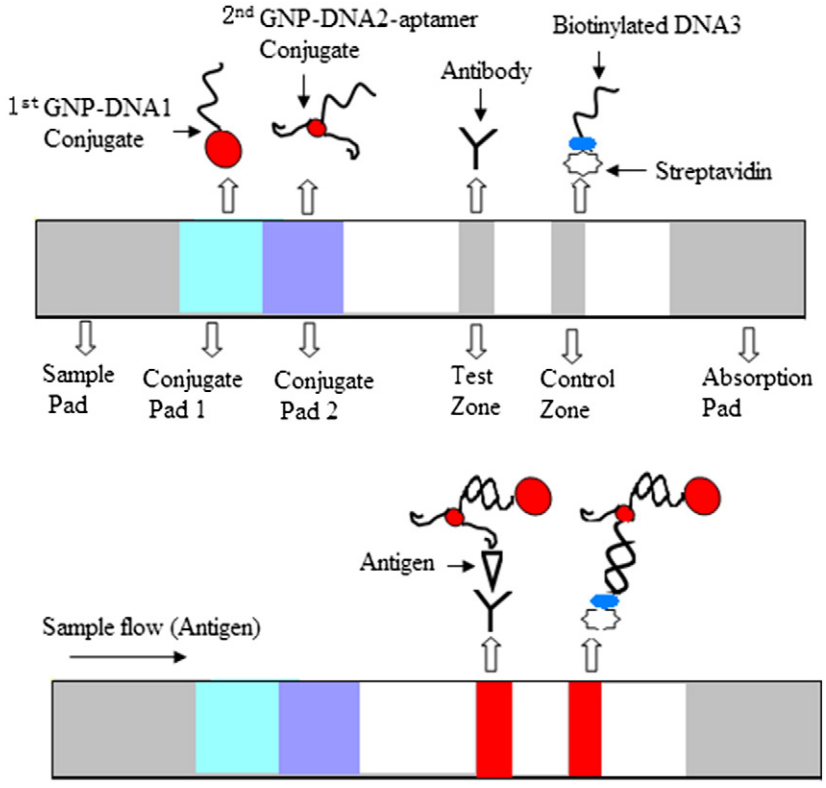

Fig. 1. Schematic illustration of the configuration and measurement principle of the dual GNP conjugates-based FLIA.

Experimental

Chemicals and materials

$Na_3PO_4 \cdot 12H_2O$, sucrose, tween 20, phosphate buffer saline (PBS, PH 7.4, 0.01 M), streptavidin, bovine serum albumin (BSA), human serum albumin (HSA), Human IgG (IgG), thrombin, dithiothreitol (DTT), triethylamine (TEA), sodium chloride-sodium citrate (SSC) buffer (pH 7.0), ethylacetate were purchased from Sigma-Aldrich. The nitrocellulose membrane, glass fiber and absorbent paper were purchased from Jiening Biotech Shanghai, Co., Ltd (Shanghai, China). Sheep polyclonal antibody to thrombin was obtained from Abcam. The aptamers and oligonucleotide probes used in this study were obtained from Takara biotechnology (Dalian, China) Co. Ltd. and have the following sequences:

Thrombin aptamer: thiolated 15-mer aptamer with polyT(20) tail, 5′-SH-$(CH_2)_6$-TT TTT TTT TTT TTT TTT TTT GGT TGG TGT GGT TGG-3′.
DNA1 oligonucleotide: 5′-SH-$(CH_2)_6$-AAA TAA TAA GTA TTT-3′.
DNA2 oligonucleotide: 5′-SH-$(CH_2)_6$-AAA TAC TTA TTA TTT -3′.
DNA3 oligonucleotide: 5′-AAA AAA AAA AAA AAA AAA AA-biotin-3′.

Preparation of GNP conjugates.

Preparation of 1st GNP-DNA1 conjugates was carried out according to previously described method [2]. The thiolated DNA1 needs to be activated before conjugation as following: 2 μL of TEA and 7.7 mg of DTT were added into a 100 μL of thiolated DNA1(1.0 OD) solution. After reacting for 1 h at room temperature, the excess DTT was removed by extraction with 400 μL of ethylacetate solution four times. A 1 mL of 5-fold concentrated 1st GNP solution was added into the activated DNA1 solution. After standing for 24 h, the solution aged by adding PBS until a final concentration of 0.01 M and then standed for another 24 h at 4 °C. The excess reagents were removed by centrifugation for 20 min at 12,000 rpm. After discarding the supernatant, the red pellets were washed, recentrifuged, and redispersed in 1 mL of an aqueous solution containing 5% BSA, 0.25% tween 20, 10% sucrose, 20 mM $Na_3PO_4 \cdot 12H_2O$.

Before preparation of 2nd GNP-DNA2/aptamer conjugates, the thiolated DNA2 and aptamer was activated respectively according to above procedure. Then, A 1 mL of 5-fold concentrated 2nd GNP solution was added into the mixture of activated DNA2 and aptamer solution (the volume ratio of activated DNA2 to aptamer solution is 3:2). The rest steps are the same as the steps described in the Section 2.3.

The 2nd GNP-aptamer conjugates were prepared using a method similar to that of preparation of 1st GNP1-DNA1 conjugates described above.

Preparation of LFIA strip

The LFIA strip is comprised of a sample pad, two conjugate pads, NC membrane, and an absorbent pad as shown in Fig. 1. The sample pad was made from cellulose fiber and was soaked with a buffer (pH 8.0) containing 0.25% triton X-100, 0.05 M tris-HCl, and 0.15 mM NaCl. Then it was dried and stored in desiccators at room temperature.

The conjugate pad 1 and conjugate pad 2 were prepared by dispensing a desired volume of 1st GNP-DNA1, 2nd GNP-DNA2/aptamer conjugate solution onto the different glass fiber pad. The pads were dried at room temperature and stored in a desiccator at 4 °C.

In order to prepare the control zone, streptavidin was used to combine with the biotinylated DNA3 according to the reported method [2]. Briefly, 50 μL of 10 OD biotinylated DNA3 was mixed with 250 μL of 2 mg mL^{-1} streptavidin and incubated for 1 h at room temperature, then 500 μL of PBS was added to the mixture. The excess biotinylated DNA3 was removed by centrifugation for 30 min with a centrifugal filter (cutoff 30,000, Millipore) at 6000 rpm. The conjugates were washed twice with 500 μL of PBS in the same centrifugal filter. The remaining solution in the filter was collected, and the solution was diluted to 500 μL by adding PBS. The test zone and control zone were prepared by dispensing antibody and the streptavidin-biotinylated DNA3 solutions in