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Label-free colorimetric aptasensor for IgE using DNA pseudoknot probe†

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The development of simple and low-cost approaches to the detection of immunoglobulin E (IgE) would provide a method for the early diagnosis and prevention of atopic diseases. The current methods of detection are generally tedious, multi-step processes and are limited by the high cost of the labeled proteins. We describe here a label-free structure-switching colorimetric method for the simple measurement of IgE using DNA pseudoknot probes and gold nanoparticles. In the absence of a target the IgE aptamer probe adopts a pseudoknot conformation that dissociates a capture probe from the unmodified gold nanoparticles. However, when IgE binds to the aptamer probe, the pseudoknot is resolved, leading to a favorable hybridization between the 3′ terminal loop of the aptamer probe and the capture probe; this induces the aggregation of the gold nanoparticles. As a result, the colorimetric IgE sensor using this structure-switching mechanism is sensitive, specific and convenient, and the assay works even when challenged with complicated biological matrixes such as vaginal fluids. The proposed method is expected to be of great clinical value for IgE detection and could be used, after appropriate design, for sensing applications of other structured aptamers.

Introduction

Immunoglobulin E (IgE), which has been linked to acute allergic reactions, is a class of immunoglobulin found only in mammals. Serum IgE is usually present in very small concentrations; abnormal amounts of IgE are a symptom of parasitic infection or other environmental issues.[1] Therefore IgE is of interest in the diagnosis of specific allergies. The most popular and well-established methods for the specific detection of IgE are adapted from traditional immunoenzymatic techniques (enzyme-linked immunosorbent assays, the radio allergo sorbent test and microarrays).[2,3] These approaches, although very reliable, are often expensive and require tedious labeling procedures and long incubation times, making them unsuitable for on-site detection. Moreover, the labeling steps that might occupy the binding site influence the affinity of the antibody.

As an alternative to antibody-based strategies, a suitable approach could be through the selection of a specific aptamer which binds to the IgE proteins. Aptamers are specific oligonucleotide sequences capable of recognizing and binding to their respective targets through the spontaneous formation of a secondary structure. Compared with monoclonal antibodies, aptamers have many advantages, including excellent stability, high affinity, ease of labeling, and simplicity of synthesis.[4] Because of their distinctive properties, aptamers have been extensively used as molecular recognition elements for bioassay applications[5–7] and various immunoassays based on aptamer probes for the detection of IgE have been developed. Commonly used methods include surface plasmon resonance,[8] fluorescence,[9] electrochemical analysis,[10] field-effect transistors,[11] and quartz crystal microbalance (QCM) techniques.[12] These approaches show promising results for the highly sensitive detection and quantification of IgE molecules. However, these methods still have a few disadvantages, such as being limited to laboratory scientific research, the use of radioactive substances, the requirement for a large sample volume, the need for washing steps before analysis, and specialized and cumbersome equipment.[13] Moreover, the detection of IgE is slow because of the laboratory-bound processes and multi-step detection reaction. Therefore sensitive, simple, and rapid methods for IgE quantitative analysis are highly desirable.

Nanomaterials have recently become extremely popular in the development of novel sensing systems with advanced functions because of their unique physical and chemical

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properties.[14] Nanoparticles have been successfully used in sensing, nanoelectronics, and catalytic applications. In particular, gold nanoparticles (AuNPs), which are detected through their plasmon resonance absorbance, have received considerable interest in bioanalytical applications because of their unique optical characteristics.[15] In addition, single-stranded DNA protects AuNPs as a result of electrostatic forces, whereas double-stranded DNA cannot electrostatically stabilize AuNPs because of its double helix geometry. Thus a combination of complementary oligonucleotides (or aptamers) and AuNPs has been used to develop simple and direct methods for the colorimetric detection of DNA,[16] proteins,[17] and other small molecules.[18] Although numerous aptamers have been reported in the construction of AuNP-based methods of analysis using naked-eye detection, only one colorimetric sensor for IgE involving the cross-linking aggregation of surface-functionalized AuNPs has been described.[19] Because the IgE aptamer contains secondary structures even in the absence of a target, it cannot be directly sensed using an unmodified strategy. Therefore exploring label-free and simple colorimetric sensors, which use unmodified AuNPs as the IgE-sensing indicator, is still challenging and appealing. We report here an alternative strategy based on label-free colorimetric detection by combining a pseudoknot DNA switch probe and AuNPs.

Experimental

Materials and apparatus

Human IgE was purchased from Abbiotec (San Diego, CA, USA). Anti-MUC1 antibody (IgG), interleukin-1, tumor necrosis factor-alpha, and interferon-gamma proteins were purchased from Abcam (Cambridge, MA, USA). Bovine serum albumin was obtained from Sigma (St. Louis, MO, USA). Psuedoknot DNA oligonucleotide was synthesized by Purigo Biotech (Taipei, Taiwan) and the other oligonucleotides were prepared at the Center for Biotechnology, National Taiwan University (Taipei, Taiwan). The sequences are as follows:

Aptamer probe (pseudoknot): GGGGCACGTTTATCCGTCCC TCCTAGTGGCGTGCCCCTTTTTTTTTTTTTTTTTAGGAGGGTA

Capture probe (DNA_C): TTTACCCTCCTATT

Random probe (DNA_R): ATTACGGTCTCAAA.

The stock solutions of 10 μM oligonucleotides were prepared in Tris–borate–EDTA buffer (90 mM Tris, 90 mM boric acid, 4 mM Na^+, 1 mM Mg^{2+}, pH 7.4) and diluted to the desired concentration with the same buffer solution. The 13 nm citrate-capped AuNPs were prepared by the reduction of $HAuCl_4$ as described previously.[20] The concentration of AuNPs was 8.5 nM, which was estimated using the Beer–Lambert law (extinction coefficient $2.7 \times 10^8\ M^{-1}\ cm^{-1}$). Ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA) and used in all experiments. UV-visible spectra were recorded on a Varian Cary 50 spectrophotometer (Varian Medical Systems, Inc., Palo Alto, CA, USA) with a wavelength range of 400–800 nm. QCM measurements were performed at room temperature using an Affinity Detection System (Taipei, Taiwan) from Asia New Technology. The photographs were taken with a Sony Xperia S camera.

Vaginal specimens were obtained as described previously.[21] Briefly, semen-free samples were collected using sterile cotton swabs. The samples were then immersed in 3 mL of phosphate-buffered saline (PBS) and vigorously mixed to evenly suspend the vaginal specimens throughout the solution. The study was approved by the Institutional Review Board of the Mackay Memorial Hospital.

IgE detection

Solutions of 100 nM psuedoknot DNA probes were incubated with different concentrations of aqueous IgE solution in 1 mM potassium phosphate buffer containing 0.3 mM KCl, 14 mM $NaCl_2$, and 0.005% Tween 20 (solution A). Simultaneously, 8 μL of 1 μM capture DNA probe were added to 200 μL of AuNP solution (4.3 nM) to form the DNA/AuNP complex (solution B). After incubation at room temperature for 60 min, solution A was mixed with solution B and the volume of the resulting solution (solution C) was adjusted to 386 μL by adding 1 mM PBS. Next, solution C was reacted for 15 min and then 14 μL of NaCl (1 M) were added to the reaction mixture for 20 min. The UV-visible spectrum of the solution was measured.

Results and discussion

Design strategy

It has been demonstrated that aptamer structures can be rationally adapted to conformation switching for the construction of biosensors.[22–24] In this way, their ligand-binding abilities are still preserved without significantly sacrificing the performance arising from the modification of non-conserved elements or the partial hybridization of the conserved elements. In particular, Freeman *et al.*[25] and Vallée-Bélisle and Plaxco[26] developed structure-switching aptamer-based sensors for small molecule targets. Inspired by their findings, we designed the pseudoknot aptamers illustrated in Fig. 1A and challenged them with AuNP-based colorimetric methods. The architecture of the probe consists of the aptamer sequence (yellow) and an additional tethered sequence (blue) complementary to the loop region of the IgE aptamer. In the absence of a target, the pseudoknot structure maintains its thermodynamically stable conformation and the additional tethered sequence does not associate with the capture strands presented on the AuNP surfaces. In the presence of a protein, however, the IgE–aptamer complex is stabilized, resulting in the dehybridization of the 3′ terminal loop. Following the conformational change from pseudoknot to hairpin aptamer, the 3′ terminal loop of the aptamer probe can associate with the capture probe and induce the aggregation of AuNPs.

Characterization of the colorimetric assay

To investigate the feasibility of the colorimetric assay, the UV-visible spectrum of the AuNP solution was measured (Fig. 1B). We treated the AuNP solution with the capture probe (DNA_C) for 30 min in the presence of the pseudoknot probe/IgE complex and pseudoknot probe alone. On the addition of salt, the former solution exhibited a color change to purple, whereas the latter

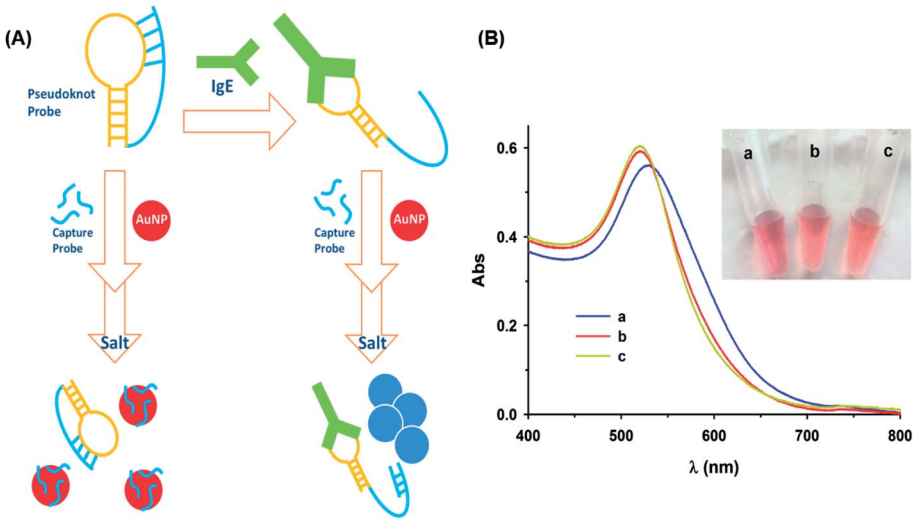

Fig. 1 (A) Schematic diagram of colorimetric sensing of IgE based on pseudoknot probes. (B) UV-visible adsorption spectra of colorimetric aptasensors under different conditions. (a) IgE/pseudoknot probe + capture probe; (b) pseudoknot probe + capture probe; and (c) IgE/pseudoknot probe + random DNA. The final concentrations of the IgE protein, the pseudoknot probe, the capture probe, and the random DNA were 5, 200, 200, and 200 nM, respectively. The inset shows the corresponding samples.

remained red (inset, Fig. 1B). It seems that the stabilization effect of the capture probes on AuNPs was insignificant in the presence of IgE, which is the initiator for the formation of the pseudoknot probe/IgE/capture probe complex. To rule out the possibility that the IgE molecule affects the stability of AuNPs, we performed a control experiment. We found that the control DNA (DNA_R) did not undergo a visible color change in the presence of the pseudoknot probe/IgE complex, suggesting that the formation of particle aggregates was mainly caused by IgE-induced hybridization events. The sensing mechanism was further confirmed by QCM measurements, which showed a notable decrease in the frequency on the addition of the pseudoknot probe/IgE complex onto the DNA_C-modified sensing surface (Fig. S1†). Taken together, these results show that the assembly of AuNPs depends on the hybridization between the capture and pseudoknot probes in the presence of IgE. The spectral absorption of the IgE aptamer/AuNP solution at 520 nm (A_{520}) is related to the amount of dispersed AuNPs. In addition, the absorbance at 600 nm (A_{600}) with the addition of IgE increased more than that at other wavelengths compared with the blank absorbance (without IgE). Accordingly, the ratio A_{600}/A_{520} was used as an indicator to express the aggregation behavior of AuNPs in subsequent experiments.

Sensitivity of IgE

The experimental conditions, including the concentration of the pseudoknot probe and the sodium ion, and the times of

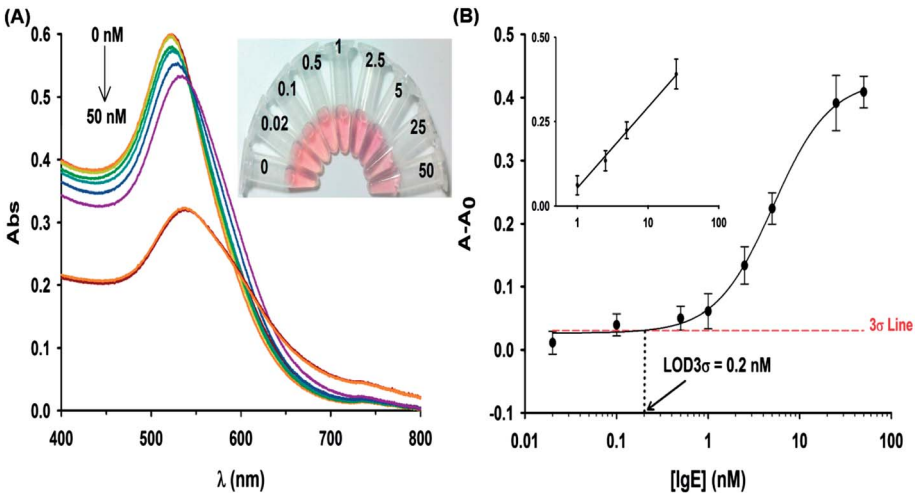

Fig. 2 Colorimetric sensing of IgE. (A) Spectra of the AuNP solutions at various concentrations of IgE (0, 0.02, 0.1, 0.5, 1.0, 2.5, 5, 25, and 50 nM). The inset shows the corresponding samples. (B) Spectral response ($A - A_0$) of the assay against increasing IgE concentrations. A and A_0 represent the spectral ratio (A_{600}/A_{520}) in the presence and absence of IgE, respectively. Error bars represent the standard deviations of three replicates. The inset shows the linear relationship between the spectral response and the concentration of IgE (from 1 to 25 nM).

incubation and response, were optimized to achieve a short analysis time and to obtain the best detection sensitivity (Fig. S2†). Under optimum experimental conditions, the aggregation of AuNPs was observed with the addition of different target concentrations. When the concentration of IgE increased, the aggregation of capture probe-stabilized nanoparticles, resulting from binding of the capture probes to the 3′ terminal sequences of the pseudoknot probes, caused a visible color change from red to violet (inset, Fig. 2A) because the aggregation led to spectral broadening and a red shift of the absorbance of the unmodified AuNPs. Fig. 2B shows a direct relationship between the aggregation of the AuNPs and the concentration of IgE. The change in the difference $A - A_0$, where A and A_0 represent the spectral ratio (A_{600}/A_{520}) in the presence and absence of IgE, respectively, increases with increasing amounts of IgE because of the formation of larger AuNP aggregates. A linear correlation was obtained in the concentration range from 1 to 25 nM with a linear coefficient $R^2 = 0.991$ (inset, Fig. 2B) and a detection limit of IgE as low as 0.2 nM (39.2 ng mL^{-1} on the basis of a molecular weight of 196 kDa) at a signal-to-noise ratio of 3σ ($n = 3$). Therefore when the IgE concentration was greater than 25 nM, it could not be accurately determined and an appropriate dilution within the linear range of the sensor was necessary in the pre-incubation step. Considering the sample volume (400 μL) obtained per measurement, this proposed aptasensor could efficiently detect down to 80 fmol of IgE. The normal physiological levels for IgE are believed to be approximately 0.85 nM, whereas concentrations in patients are significantly increased by at least 10-fold (approximately 8.5 nM),[27] therefore our method has the potential to be used in routine clinical applications. In comparison with previously reported methods, our approach avoids constructing complicated aptasensors and shows better or comparable sensitivity toward IgE molecules.[28–31] Of note, however, compared with the sticky-end pairing-based colorimetric aptasensor reported by Wu *et al.*,[19] our approach is less sensitive. This existing assay, however, requires slow, multi-step preparation processes of nanoparticle–aptamer conjugates that take 2 days, thus our approach is more convenient, faster, and cheaper.

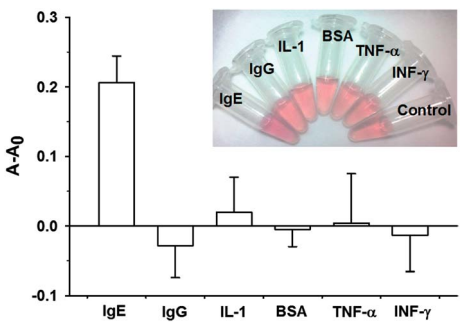

Fig. 3 Selectivity of the pseudoknot probe toward IgE. The inset shows the samples containing the probe with different proteins. The concentration of IgE was 5 nM, and the concentration of each of the other proteins was 10 nM.

Selectivity of colorimetric detection

The selectivity of this AuNP sensor for IgE towards other physiologically related molecules was examined by a visual assay. As shown in the inset of Fig. 3, only IgE (5 nM) caused a red to purple color change, whereas the solutions of other proteins that were tested remained red at a concentration of 10 nM. IgE led to a notable spectral ratio change, whereas the other proteins caused slight ratio changes that overlapped with the background signal, indicating that only IgE effectively triggered the aggregation of AuNPs. Therefore this proposed aptasensor shows the expected selectivity. In addition, further experiments were performed in diluted clinical samples, and the results showed great potential for practical applications (Fig. S3†).

Conclusions

We have developed a colorimetric biosensor strategy based on a label-free pseudoknot switch probe for the simple, but effective, sensing of IgE with high sensitivity and selectivity. This assay gave a detection limit of 0.2 nM for instrumental detection and a linear range up to 25 nM. Moreover, this method can identify IgE spiked into vaginal fluids, providing a good option for this area of clinical analysis. Compared with existing IgE detection techniques, this sensing approach has the advantages of speed, simplicity, and ease of operation. In addition, this method could be further improved by modifying the probe geometry or length. This aptasensor is facile and cost effective as it avoids the modifying, immobilizing, and labeling processes that result in relatively complicated operation and high cost. Given these features, this proposed colorimetric assay could further facilitate the analytical applications of other aptamers with hairpin-based structures with appropriate probe designs.

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