

Aptameric enzyme subunit for homogeneous protein sensing

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ABSTRACT

We have developed an aptameric enzyme subunit (AES) for protein sensing. AES is composed of an enzyme-inhibiting aptamer and a target molecule-binding aptamer and it can allosterically control the corresponding enzyme activity by binding target molecules. Here, we applied AES to immunoglobulin E (IgE) sensing. We split the thrombin-inhibiting aptamer into two oligonucleotides and then connected IgE-binding aptamer and its complementary strand to the split thrombin-inhibiting aptamer. The hybrid of these two oligonucleotides inhibited thrombin enzymatic activity and it was decreased in the presence of IgE. We were able to detect IgE by using the AES in homogeneous assay and detection limit was 250 nM.

INTRODUCTION

Aptamers [1, 2] have some advantages over antibodies and one of them is the structure change of aptamers can be designed by the straightforward hybridization rules. Recently, homogeneous sensing systems which used structure change of aptamers have been reported [3]. Those sensor systems mainly use fluorescence resonance energy transfer (FRET) to detect their target molecules. We have reported aptameric enzyme subunit (AES) which can detect target molecules without separation steps [4, 5]. AES is composed of an enzyme-inhibiting aptamer and a target molecule-binding aptamer and it can allosterically control the corresponding enzyme activity by binding target molecules. AES enables the homogeneous detection of target molecules by measuring enzymatic activity therefore highly sensitive detection can be expected compared with FRET-based techniques. Moreover, AES can be constructed easily because AES can be made by just oligonucleotide which does not contain any modification. Therefore AES would archive highly sensitive, easy and rapid assay.

Here, we describe design strategy of an AES which can response to the target protein. We used the thrombin-inhibiting aptamer [6] as an enzyme-inhibiting aptamer and the IgE-binding aptamer [7] as a target molecule-binding aptamer and connected them to construct an AES which can response to IgE.

RESULTS AND DISCUSSION

The thrombin-inhibiting aptamer was split into two oligonucleotides at 3' T-T loop site on G-quartet structure. The one of the split oligonucleotide was attached with the IgE-binding aptamer and the other strand was attached with 9-mer complementary strand of the IgE-binding aptamer. We expected that these oligonucleotides can be hybridized each other to fold native thrombin-inhibiting aptamer structure which can inhibit thrombin enzymatic activity. However, these oligonucleotides were not able to inhibit thrombin enzymatic activity (data not shown). It means that 9-mer complementary strand of the IgE-binding aptamer is not enough to hybridize with other strand. Therefore we added 4-bp linker DNA between the thrombin-inhibiting aptamer site and the IgE-binding aptamer site to help hybridization each other. The sequences and presumed structure of the AES is shown in Figure 1. The oligonucleotide which contains the IgE-binding aptamer is named as 5' AES and the other strands is named as 3' AES.

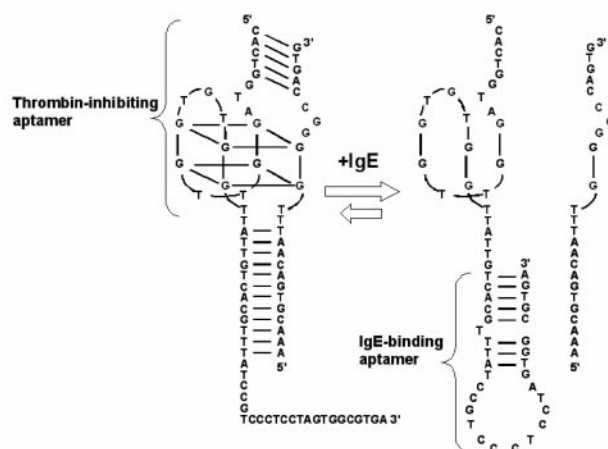


Fig. 1 Presumed structure of the AES for IgE sensing and its structure change by addition of IgE.

The thrombin enzymatic activities were determined by measuring the clotting time of fibrinogen solution. The thrombin-inhibiting effects of the AES are shown in Figure 2. The 5' AES and 3' AES did not inhibit thrombin enzymatic activity alone (data not shown), but the hybrid of the 5' AES and 3' AES inhibited thrombin enzymatic activity (lane 5). The inhibitory activity of the thrombin-

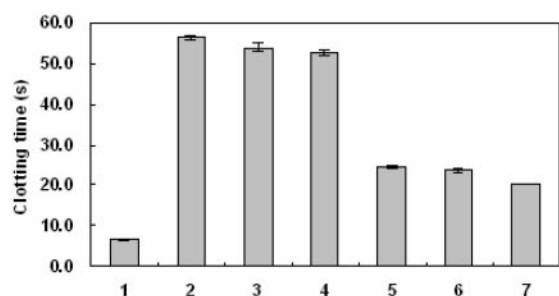


Fig. 2 Inhibition of clotting by addition of the thrombin-inhibiting aptamer and the AES. Those experiments were performed using 250 nM thrombin-inhibiting aptamer, 250 nM AES, 250 nM IgE, 250 nM BSA, 54 nM thrombin and 1.7 mg/ml fibrinogen at 37°C. Lane 1; thrombin only, lane 2; thrombin-inhibiting aptamer, lane 3; thrombin-inhibiting aptamer with BSA, lane 4; thrombin-inhibiting aptamer with IgE, lane 5; AES, lane 6; AES with BSA, lane 7; AES with IgE. Clotting times are the average of three experiments and error bars are indicated.

inhibiting aptamer did not change by addition of IgE compared with it in the presence of BSA (lane 2, 3, and 4), but the inhibitory activity of the AES decreased by addition of IgE (lane 5, 6, and 7). The clotting time is changed from 24 s to 20 s). Those results indicate that the AES was able to respond to IgE selectively

We made a calibration graph using 250 nM AES and 0.34 mg/ml fibrinogen, as shown in Figure 3. Although thrombin inhibition was hardly influenced by the addition of BSA, it did decrease with IgE in a concentration-dependent manner. Therefore, we can say that IgE was selectively detected by using the AES and the lower detection limit was 250 nM.

The sensitivity of the AES was not higher than ELISA because we used the thrombin-inhibiting aptamer as an enzyme-inhibiting aptamer. We determined thrombin enzymatic activity by measuring clotting time which can not amplified the detection signal effectively. The thrombin enzymatic activity also can be measured by using peptide modified with fluorescence, because thrombin is one of serine protease. However thrombin-inhibiting aptamer can not inhibit the binding of peptide to active site of thrombin because the thrombin-inhibiting aptamer binds to fibrinogen binding site of thrombin. However the design strategy of the AES described here can be applied to any enzyme-inhibiting aptamers because many aptamers use intramolecular hybridization to fold their three-dimensional structures. It means that aptamers can be split at the stem-loop forming site easily. There are some other reports that describe the splitting aptamers into two oligonucleotides that hybridize when their target molecules are present [8]. In this study, we used a thrombin-inhibiting aptamer; if a more suitable enzyme-inhibiting aptamer for AES is used, the sensitivity of the sensing system can be improved.

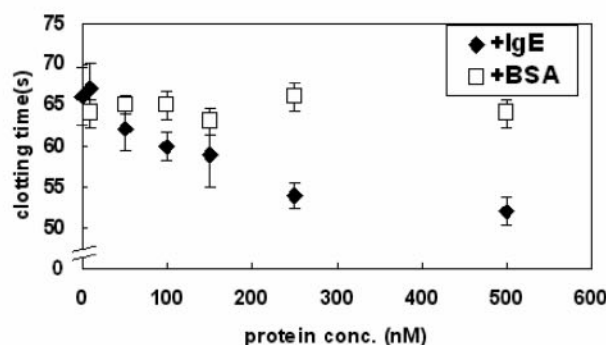


Fig. 3 Calibration graph of IgE using the AES. Those experiments were performed using 250 nM AES, 54 nM Thrombin and 0.34 mg/ml fibrinogen at 37°C. Clotting times are the average of three experiments and error bars are indicated

CONCLUSION

We constructed an AES which can detect IgE by measuring thrombin enzymatic activity. Using the AES, we can detect IgE without separation steps and lower detection limit was 250 nM. We have already developed AESs for adenosine and target DNAs sensing [4, 5]. Therefore AES enable us to detect many target DNAs, RNAs, low-mass molecules, and proteins in a common assay.

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