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Fluorescence aptasensor based on competitive-binding for human neutrophil elastase detection

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ABSTRACT

To our knowledge, we report the first fluorescence aptasensor for detecting human neutrophil elastase (HNE) in homogeneous solution. The biosensor contains a short DNA scrambled sequence strand (SS) complementary to part of the aptamer sequence or the loop of molecular beacon (MB). The aptamer-HNE recognition event involves competition between the molecular beacon and loose HNE aptamer for the binding the short DNA strand. The new biosensor can detect as little as 0.34 nM of HNE, and the response is linear in the tested concentration range of 0.34–68 nM with the detection limit of 47 pM.

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1. Introduction

Neutrophil granulocytes are primary antimicrobial effector cells of the innate immune system and contribute to the first line of defence against infectious agents or nonself substances that penetrate the body's physical barriers [1]. The human neutrophil elastase (HNE), a serine proteinase, is the principal enzyme released from neutrophils [2]. It is capable of solubilising fibrous elastin, cartilage proteoglycans, several collagens and fibronectin, hence facilitating the migration of cells to inflammatory regions. HNE is thought to physiologically participate in disease resistance by facilitating the degradation and phagocytosis of pathogenic bacteria [3]. Uncontrolled activity of HNE has been shown to contribute to the pathogenesis of rheumatoid arthritis [4], chronic obstructive pulmonary disease [5], adult respiratory distress syndrome [6], glomerulonephritis [7], and chronic and burn wounds [8,9].

Lin et al. [10] identified a specific high-affinity DNA ligand targeted to HNE using an in vitro selection technique (SELEX). The DNA ligand folds into a G-quartet structure with duplex ends. According to Davis's work [11], the fluoresceinated DNA ligand equally effective as an anti-HNE antibody in detecting HNE can be useful in diagnostic applications based on flow cytometry. Charlton et al. [12] applied the DNA aptamer ligand to the field of diagnostic imaging.

Fluorescence spectroscopic methods offer high sensitivity and selectivity and applicability in nonseparation detection and in situ monitoring [13–16]. Nutiu and Li [17], for example, described aptamer-based fluorescent reporters for detecting for thrombin over the range of 10 nM–1.0 μM that a structural switch releases a dabcyl-labeled nucleotide strand from the fluorophore-labeled aptamer. Li et al. [18], on the other hand, prepared a molecular aptamer beacon (MAB) for recognizing the thrombin producing significant fluorescent signal change, which is attributed to a significant conformational change at low concentration (112 pM). Lerga and O'Sullivan [19] developed thrombin MAB labeling fluorescein and coumarin to selectively detect Ca^{2+} and Mg^{2+} with detection limit (40 μM) for determination of total water hardness in tap and bottle water. Heyduk and Heyduk [20] introduced a fluorescent assay involving thrombin-induced coassociation of two aptamers with analyte resulted in bringing the two short fluorophore-labeled oligonucleotides into proximity. Change of fluorescence resonance energy transfer was even at the lowest thrombin concentration tested (50 pM).

In our research, we proposed a sensitive method for HNE analysis in homogeneous solution. The molecular beacon was designed by appending fluorescein to 5′-ends and dabcyl quencher to 3′-ends. The HNE aptamer was free-labeled in order to retain the bioactivity. The short DNA scrambled sequence was complementary to the binding sites of the HNE aptamer or the loop of molecular beacon. Our biosensor involves aptamer-HNE recognition, competition between the molecular beacon and loose HNE aptamer for binding the short DNA strand, and monitoring the extent of

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competition through highly sensitive fluorescence detection of the captured HNE.

2. Experimental

2.1. Materials and reagents

HNE from human plasma (SERVA Electrophoresis GmbH, Heidelberg, Germany) was dissolved in a storage buffer (50 mM Tris–HCl, 50 mM NaCl, 3.0 mM KCl, 1.0 mM $MgCl_2$, pH 7.5) to yield 3.39 μM solutions. This standard was divided into 100 μL aliquots and stored at −20 °C. Immediately before use, each aliquot was warmed to 4 °C and diluted with a storage buffer solution to the required concentration. HNE concentration mentioned in our work was the molarity of HNE storage solution before fluorescence measurements.

Oligonucleotides used in the study were customer-designed and synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) as follows.

The HNE aptamer: 5′-TAGC GATA CTGC GTGG GTTG GGGC GGGT AGGG CCAG CAGT CTCGT,
The molecular beacon (MB): 5′-fluorescein-CCTA GCGC GTGG GTTG GGGC TAGG-Dabcyl,
Four scrambled sequence (SS):
S12: 5′-CCCA ACCC ACGC; S14: 5′-GC CCCA ACCC ACGC; S16: 5′-CCGC CCCA ACCC ACGC; S19: 5′-TAC CCGC CCCA ACCC ACGC.

Ultrapure water used to prepare all of the solutions was obtained through a Nanopure Infinity Ultrapure water system (Barnstead/Thermolyne Corp., Dubuque, IA) with an electrical resistance larger than 18.3 MΩ. All buffer solutions and ultrapure water were sterilized and used throughout experiments. Sodium chloride, potassium chloride, human serum albumin (HSA), magnesium chloride and Tris–HCl were purchased from China National Medicines Co. Ltd. (Beijing, China).

2.2. Fluorescence measurements

If not otherwise specified, the following concentrations of oligonucleotides were used for fluorescence measurements: 40 nM for aptamers, 40 nM for the SS and 40 nM for the MB. MB was dissolved in storage buffer to form hairpin duplex, while HNE aptamer and SS were dissolved in ultrapure water to keep flexible structure. The binding buffer (pH 7.5) consisted of 100 mM Tris–HCl, 850 mM NaCl, 16 mM KCl, 5.0 mM $MgCl_2$ and 0.12% HSA (wt.%).

To perform the fluorescence experiments, 50 μL HNE solution of a specific concentration and 100 μL binding buffer were incubated with 50 μL HNE aptamer for 45 min. After the addition of 50 μL of a SS solution, the mixture was incubated for 10 min at 10 °C and then added 50 μL MB solution.

All fluorescence measurements were performed in 50 mM Tris–HCl (pH 7.5), 300 mM NaCl, 6.0 mM KCl, 2.0 mM $MgCl_2$ and 0.02% HSA (wt.%). All fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrometer (Hitachi Ltd., Japan) controlled by FL Solution software to fit a curve and determine the peak height. A quartz fluorescence cell with an optical path length of 1.0 cm was used. The excitation was made at 495 nm with a recording emission range of 500–600 nm. All excitation and emission slits were set at 5 nm. The fluorescence spectrum of all mixtures was recorded at 10 ± 2 °C.

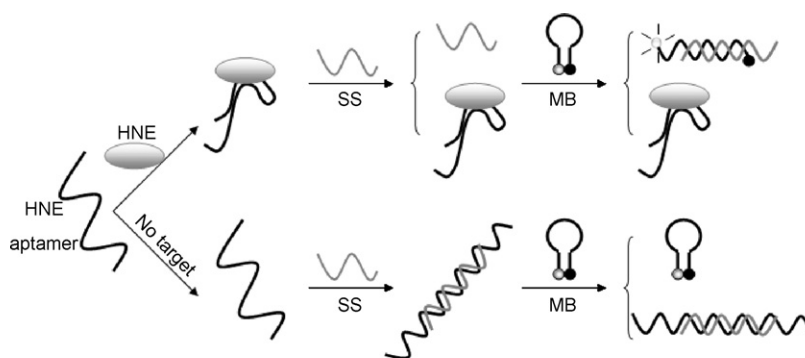
3. Results and discussion

3.1. Experimental principle

Scheme 1 illustrates the concept of the competitive-binding process coupled with fluorescence increase in this design. Before the addition of target, HNE aptamer (the long black coil) kept flexible structure in the blank experiment. SS (the short grey coil) was introduced in this homogeneous solution, and naturally binds to the aptamer. When MB (the short coil with two rotundities) was introduced, the hybridization of SS with MB would not take place immediately, which kept the fluorescence off. In the presence of HNE (the ellipse), the flexible structure of aptamer was transformed into a HNE-aptamer duplex assembly with G-quartet structure. Upon addition of SS and MB, the corresponding complementary sections of SS would hybridize with MB producing a strong concentration-dependent fluorescent signal.

3.2. Fluorescence increase of HNE binding

Fig. 1 demonstrates fluorescence spectra of MB solutions, a mixture of aptamer/SS/MB, an assembly of aptamer/HNE/SS/MB and a duplex of SS/MB. The sample containing MB only (Fig. 1, curve a) shows rather low fluorescence intensity for its hairpin structure keeping the fluorophore and the quencher into proximity. A slight fluorescence increase was observed when the MB was added into solution containing aptamer and SS (curve b). It can be interpreted as that a small quantity of MB captured SS dissociated from aptamer/SS. In the presence of HNE (17 nM, curve c), a significant fluorescence intensity increase (102%) was recorded at the emission peak around 518 nm. Binding of target proteins forces the aptamers to undergo a conformational change, while MB opens its hairpin structure leading to an increase in fluorescent signal. When



Scheme 1. Schematic representation of the aptamer-based HNE sensor. HNE: the ellipse; unmodified aptamer: the long coil; DNA scrambled sequence (SS): the short coil; DNA molecular beacon (MB): the short coil with two rotundities.

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