Analytical Biochemistry 525 (2017) 78–84

Contents lists available at ScienceDirect

Analytical Biochemistry

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

High affinity truncated DNA aptamers for the development of fluorescence based progesterone biosensors

Hani A. Alhadrami [a, 1], Raja Chinnappan [b, 1], Shimaa Eissa [b], Anas Abdel Rahamn [c], Mohammed Zourob [b, c, *]

[a] Faculty of Applied Medical Sciences, King Abdulaziz University, Post Office Box 80402, Jeddah 21589, Saudi Arabia
[b] Department of Chemistry, Alfaisal University, Al Zahrawi Street, Al Maather, AlTakhassusi Rd, Riyadh 11533, Saudi Arabia
[c] King Faisal Specialist Hospital and Research Center, Zahrawi Street, Al Maather, Riyadh 12713, Saudi Arabia

ARTICLE INFO

Article history:
Received 1 January 2017
Received in revised form
12 February 2017
Accepted 20 February 2017
Available online 24 February 2017

Keywords:
Aptamers
Progesterone
Hormones in water

ABSTRACT

Aptamers have shown a number of potential applications in sensing and therapeutic due to the high affinity and specificity towards their target molecules. Not all the nucleotides in the full length aptamers are involved in the binding with their targets. The non-binding domain of the aptamer may affect the binding affinity of the aptamer-target complex. Mapping the aptamer binding region could increase the affinity and the specificity. In this paper, we designed aptamer-based fluorescence sensors from a truncated progesterone (P4) aptamer. Then, fluorescein and quencher labelled aptamer complementary oligonucleotide sequences were hybridized to the truncated aptamer at different sites to form duplex structures. We used fluorescence-quencher pair displacement assay upon progesterone binding for the determination of P4. One of the truncated sequences has shown high binding affinity with 16 fold increase in the dissociation constant, K_d (2.1 nM) compared to the original aptamer. The aptasensor was highly selective for P4 against similar compounds such as 17-β estradiol, bisphenol-A and vitamin D. The sensor has been applied for the detection of P4 in spiked tap water and in urine samples showing good recovery. This new developed aptamer-based fluorescence biosensor can be applied in food, pharmaceutical, and clinical industries.

1. Introduction

Food products and drinking water contamination with endocrine-disrupting chemicals (EDCs) may have a serious effect on hormonal functions such as metabolism, biosynthesis, etc [1]. Progesterone (P4) is a small hydrophobic steroid hormone secreted from corpusluteum, which plays a major role in mammalian pregnancy, animal growth and development. Progesterone level is used as an indicator for early pregnancy. Many clinical assays are currently used to measure hormonal levels in a variety of body fluids. The progesterone concentration is 1 ng/ml in serum during the pre-ovulation period, 20 ng/ml in the mid cycle and more than 300 ng/ml at the pregnancy period. Elevated levels of P4 leads to headache, breast tenderness, stomach upset, constipation, diarrhea, body pain, tiredness, virginal discharge and urinal infections [2]. Consumption of high levels of progesterone in cow milk may case breast and lung cancers [3,4] and it affect the gonadotropin (GnRH) releasing hormone secretion in males [5]. When high amount of P4 is consumed, the body retains certain amount and the rest is released to the environment as waste. Therefore, it is highly important to monitor P4 levels in environmental and clinical samples.

Several methods are applied for the detection of P4. Instrumental analysis methods such as high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC-MS) are highly sensitive. However, they need well-trained operators, high cost and not suitable for field applications. Immunoassays like enzyme linked immunosorbent assay (ELISA), radioimmune assays, electrophoresis-chemiluminescence and non-competitive audiometric assays are used for the detection of P4 in different samples [6–8]. However, the limited stability and high cost of the immunoassays are still major challenges.

* Corresponding author. Department of Chemistry, Alfaisal University, Al Zahrawi Street, Al Maather, AlTakhassusi Rd, Riyadh 11533, Saudi Arabia.
E-mail address: mzourob@alfaisal.edu (M. Zourob).
[1] Equal Contribution.

http://dx.doi.org/10.1016/j.ab.2017.02.014
0003-2697/

Recently, there is an increasing interest towards aptamers as alternatives to antibodies. Aptamers are widely used for sensor development for the detection of various compounds for clinical, food and environmental applications. Aptamers bind their target specifically with high affinity (K_ds in the nanomolar to picomolar range). They can be synthesized easily, highly stable and their production cost is very low. Aptamers are single stranded DNA (ssDNA) or RNA, consisting typically of 40–100 nucleotides. At suitable conditions, aptamers form unique 3D structure due to the intra molecular forces of attractions such as hydrogen bonding, hydrophobic and Wan der walls interactions between the nucleotides. This unique secondary or tertiary structures of the aptamer forms a binding packet that fits the target molecule and form a stable target-aptamer complex. Aptamers for various analytes have been reported including metal ions [9], proteins [10], bacteria [11], viruses [12] and small molecules [13–15]. The in vitro selection of aptamers are usually screened from a pool of DNA or RNA libraries using systematic Evaluation of Ligands by Exponential Enrichment (SELEX) method.

The goal of applying aptamers in bioanalysis is to report the presence of target without any complicated steps. Fluorescence detection is one of the commonly used analytical ways using aptamers. The aptamer results from the classical SELEX does not usually exhibit intrinsic fluorescence. The fluorescent molecule is introduced at the position where the aptamer undergo the target induced conformation change [16,17] or fluorescent nucleotides in the library pool are introduced during the in vitro selection of aptamer [17]. However, it is not straight forward to identify the region where the confirmation change occurs. Moreover, if there is no significant change in the fluorescence intensity upon target binding, the sensitivity of the detection assay will be low. Therefore, it is important to know the target binding region where the aptamer undergo conformation change upon target binding. By truncating the non-binding region, the aptamer may form better conformation to make a stronger aptamer-target complex. Many approaches have been used for truncating the aptamers. For example, the secondary structure of the Tat HIV protein aptamer can be split into two parts. A molecular beacon (in which the fluorophore and quenchers are attached to both ends) and a complementary sequence to the stem of the beacon. In the presence of target, the beacon undergoes conformation change which leads to a physical separation of the fluorophore and quencher resulting in a change in the fluorescence signal [18].

Here, we have developed a fluorescence-based aptasensor for the detection of P4 via structure switching mechanism. In this study, we designed truncated aptamers/DNA duplex structures by annealing fluorescein-labelled (FDNA) and quencher-labelled DNA (QDNA) complementary sequences at different sites. Originally, in the designed duplex structures the fluorophore and quencher will be in close contact to each other leading to minimal fluorescence intensity. However, in the presence of P4, an increase in the fluorescence intensity is recorded due to the conformation change of the aptamer upon target binding which leads to FDNA and/or QDNA dissociation. This is due to the favourable formation of the aptamer-P4 complex than the DNA duplex form. Knowing the minimal binding sequence within the aptamer, a biosensing platform for the detection of P4 was established.

2. Experimental

2.1. Materials and methods

Progesterone, 17-β estradiol, bisphenol A, vitamin D, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris base), sodium chloride, ethylenediaminetetraacetic acid (EDTA), sodiumazide, potassium phosphate, sodium hydroxide, sodium chloride, potassium chloride, urea, sodium phosphate, hydrochloric acid dimethyl sulphoxide, DMSO and magnesium chloride were purchased form Sigma-Aldrich (Saint-Louise, MO, USA). HPLC purified labelled and unlabelled oligonucleotides (Table 1) were purchased from Metabion International (Planegg, Germany). A solution of 50 mM tris-HCL (pH 7.5), 150 mM NaCl and 5 mM $MgCl_2$ is used as binding buffer. The stock solution of P4, 17-β estradiol, bisphenol A and vitamin- D was prepared in DMSO and further dilutions were made in binding buffer. The DNA were dissolved in ultra pure Milli-Q water to make the stock solutions and stored at −20 °C until further use. The DNA solutions used in the experiments were diluted in binding buffer. The fluorescein-labelled oligonucleotides were protected from the light while performing the experiments.

2.2. Fluorescence measurements

The fluorescence of the fluorescein labelled complementary aptamer oligonucleotides and the aptamer-beacon has been measured using Nanodrop ND3300 fluorospectrometer (Thermo Scientific, Canada) and Molecular device F5 fluoromax microtiter plate reader (Sunnyvale CA, USA) using 96 well fluorescence plates. The samples were excited at 480 ± 10 nm and the emission was monitored at 525 ± 10 nm. All the measurements were recorded in binding buffer at room temperature.

2.3. Designing the aptamer sequences

The truncated aptamers were designed based on the secondary structure of PG13 full length aptamer selected in our previously published work [15]. The secondary structure was obtained from the mfold software. Two designs were done; displacement of a complementary sequence and molecular beacon. The complementary sequences, 5′-fluorescein labelled and 3′-dabcyl quencher labelled oligonucleotides were designed for the fluorescence-based competitive displacement assays in which the fluorescein quencher pair are in close contact upon hybridization with the truncated aptamer. The PG13 aptamer was truncated into two different domains as shown in Table 1 The aptamer- beacon was designed from the original full length aptamer with addition of few nucleotides either 5′ or 3′ end. Aptamer-beacons are similar to molecular

Table 1
Original and truncated aptamers sequences.

Name	Sequences
Pg13	GCATCACACACCGATACTCACCCGCCTGATTAACATTAGCCCACCGCCCACCCCCGCTGC
PG13T1	GCATCACACACCGATACTCACCCGCCTGAT
PG13C1	Flu-5′-CGGTGTGTGATGC
PG13C2	GGCGGGTGAGTAT-3′-Dabcyl
PG13T2	GATTAACATTAGCCCACCGCCCACC
PG13C3	Flu-5′-CTAATGTTAATC
PG13C4	TGGGCGGTGGG-3′-Dadcyl
PG13MB	Flu-5″CAGCCCACCGCCCACCCCCGCTG-3″-Dabcyl