

Aptamer base nanobiosensor for stress hormone measurement

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Abstract

Cortisol plays an important part in the physiological procedures. Observing cortisol concentration as a stress indicator demonstrates physiological mode for diagnosis goal. The mechanism of presented nanobiosensor is LSPR and performed based on the nucleic acid aptamer functionalized with gold nanoparticles which has specific interaction with cortisol. The interaction of aptamer particular measurement of cortisol was accomplished over the concentration from 0.05 -0.2 $\mu\text{g}\cdot\text{mL}^{-1}$ within 20 minutes. The detection limit is estimated to be 0.02 $\mu\text{g}\cdot\text{mL}^{-1}$.

Keywords: Aptamer, silver nanoparticles, Nanobiosensor, cortisol.

1. Introduction

Serious and long-term stresses have irreversible impacts on human mental wellbeing [1]. Stress symptoms are classified into four categories: physical, behavioral, emotional and cognitive [2]. Physical side effects of stress incorporate stomach related issues, migraine, back torment, acid reflux, neck spasms, fast and shallow breathing, quick pulse, cold hands and Feet restlessness, tinnitus, dizziness, fatigue, constipation, muscle tension and diarrhea [3]. Emotional symptoms includes irritability, nervousness, anxiety, tiredness and boredom, simply crying, stress, anger, loneliness, clumsiness,

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sadness, depression, mood swings, feelings of helplessness, and feelings of helplessness [4]. Cognitive symptoms include difficulty in thinking, inability to make decisions, constant worry, forgetfulness, self-criticism, loss of creativity, difficulty concentrating, pessimism and high expectations of others [5].

Aptamers have interesting properties that make them better than other biorecognition part for example, antibodies [6]. These properties include very small size compared to antibodies, high stability, structure reversibility against Types of physical changes such as temperature, pH, the presence of metal ions, the ability to connect to a wide variety of purposes, the possibility of making different and adding various functional and chemical groups, easy storage, easy stabilization on different surfaces, facile transport and synthesis methods, High connection tendency, low separation speed and high specificity [7]. Silver nanoparticles are act as transducers in this nanobiosensor system. The interaction of aptamer with cortisol is evaluated as a signal in a linear correlation with concentration. In the presence of different concentration of cortisol, the absorbance of silver nanoparticles increased.

2. Materials and methods

2.1 Reagents

The further process was not applied to analytical grade chemical substances. for making all aqueous solutions for eliminating the side effect of ions, deionized water (MilliporeSigma AFS®D) was used. H₂AuCl₄, β-estradiol, Cortisol, progesterone, testosterone and trisodium citrate purchased from Merck (Germany).

2.2 Aptamer

The single-strand DNA aptamer sequence which designed specifically for this study is 5'-ATGGGCAATGCGGGGTGGAGAATGGTTGCCGCACTTCGG-3'-Thiol. - DNA synthesis, purification by HPLC and quality control MALDI-TOF was performed in Metabion international AG (Steinkirchen, Germany).

2.3 Synthesis the gold nanoparticles

Gold nanoparticles was synthesized based on some modifications in the Turkevich method procedure [8]. Colloidal gold nanoparticles were synthesized using trisodium citrate as a reducing agent. Briefly, 50 ml of 0.25 mM gold chloride (H₂AuCl₄) was heated using and vigorous stirring 34.0 mM (1.0 wt.%) trisodium citrate (Na₃Ct) solution was added rapidly. The mixture was shaken for 1 min vigorously. The resultant solution was incubated at 4 °C in the dark overnight [9]. The gold nanoparticle was applied without further purification for cortisol detection. The gold nanoparticle attached to aptamer via thiol group which placed in 3' end.

2.4 Apparatus and sample preparation

The spectrums are the mean of three replicates of experiment.

- **Absorbance** The ND-2000C Nano Drop (Thermo Scientific, USA) was applied for UV visible absorption measurement. the ranges of wavelength was 200nm 800nm. The volume of samples was 4μl.

- **Microscopic imaging** (FE-SEM) microscope (MIRA3; TESCAN, Brno, Czech Republic) was applied for nanoparticle investigation. The Gold thin film was deposited on the glass surface by the magnetron sputter ASSC (Adeco, Iran). The sputtering procedure was running by argon as sputter gas and gold target with 3.8 diameters. The glass was placed with no tilt. Distance from the target was set as 19 cm. Pressure in the chamber was set from 0.15 Pa to 4 Pa. The power and deposition time were set as 100 W set and 1800s respectively.

3. Results and discussion

Mfold web server was applied for prediction of secondary conformation and calculation of thermodynamic folding parameters was done. The predicted secondary structure was shown in Figure 1. Thermodynamic parameters such as Gibb's entropy, free energy, melting temperature and enthalpy were calculated and presented in Table 1. The Figure 1 showed predicted model, calculated based on thermodynamic parameters. Explicit diagram of this model showed the stem loop structure of this aptamer. The secondary structure of middle part, specified for cortisol recognition, formed clearly.

In Figure 2 dots showed the superposition of whole possible foldings by p% of ΔG_{mfe} , where P is the max% deviation from ΔG_{mfe} . The suboptimality level is shown in color.

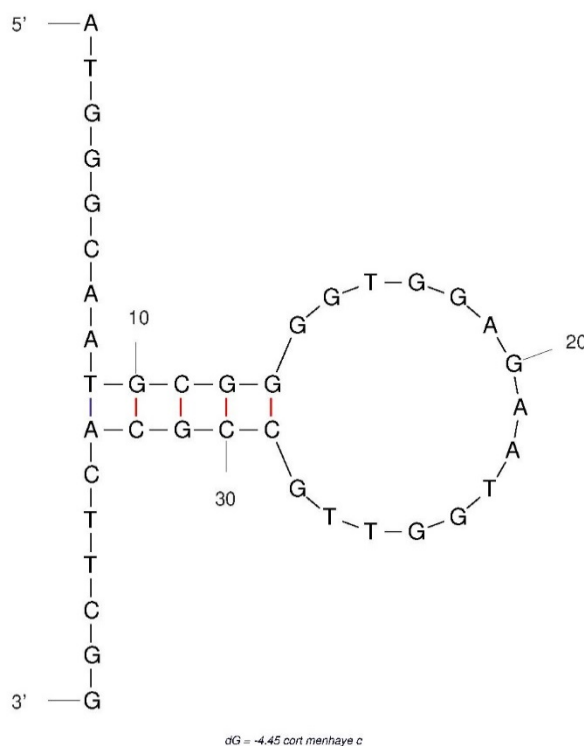


Figure 1. The predicted model of aptamer based on thermodynamic calculation.

Table 1. the thermodynamic calculation of the aptamer

$$\Delta G = -4.45$$

Structural element	δG	Information
External loop	-0.45	14 ss bases & 1 closing helices.
Stack	-1.45	External closing pair is T ⁹ -A ³³
Stack	-2.24	External closing pair is G ¹⁰ -C ³²
Stack	-2.17	External closing pair is C ¹¹ -G ³¹
Stack	-1.84	External closing pair is G ¹² -C ³⁰
Helix	-7.70	5 base pairs.
Hairpin loop	3.70	Closing pair is G ¹³ -C ²⁹

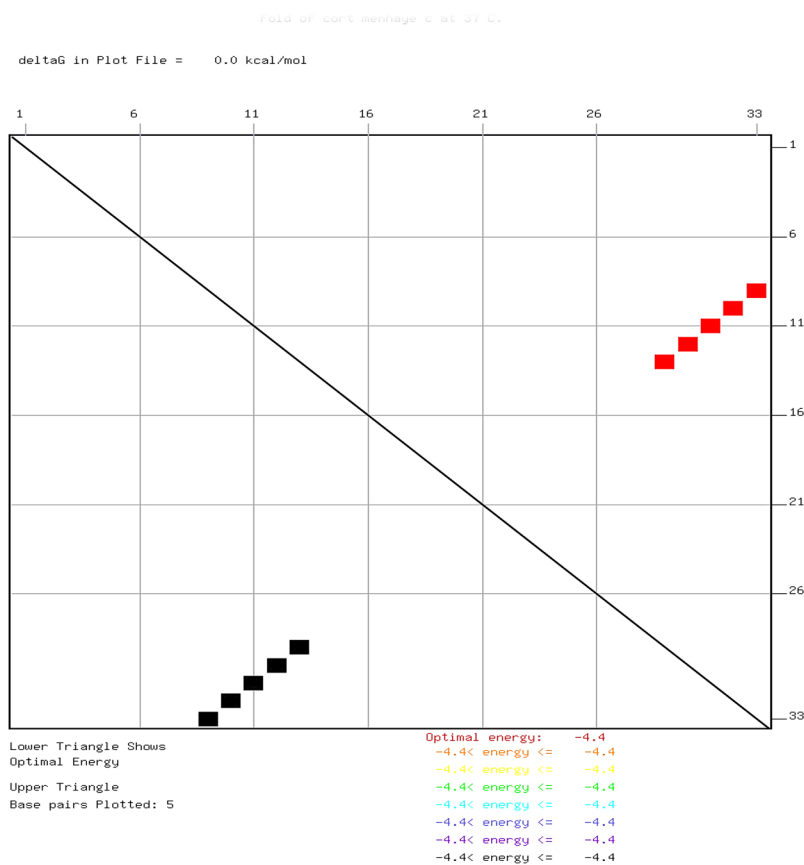


Figure 2. Energy dot plot for Aptamer.

In Figure 3 the morphology of gold nanoparticles demonstrated. The size of AuNPs was 75 nm. SEM images of gold nanoparticle show monodispersed mode and the similarity in nanoparticle shape is observed in the Figure 3.

UV-vis absorbance spectra demonstrated in Figure 4. Show the increasing intensity of absorbance in the presence of cortisol. The selectivity assay of colorimetric detection of cortisol using AuNPs and aptamers is performed via β -estradiol, Testosterone and progesterone the changes in spectra weren't significance. The interaction of aptamer particular measurement of cortisol was accomplished over the concentration from 0.05 -0.2 $\mu\text{g}\cdot\text{mL}^{-1}$ within 20 minutes. The detection limit is estimated to be 0.02 $\mu\text{g}\cdot\text{mL}^{-1}$.

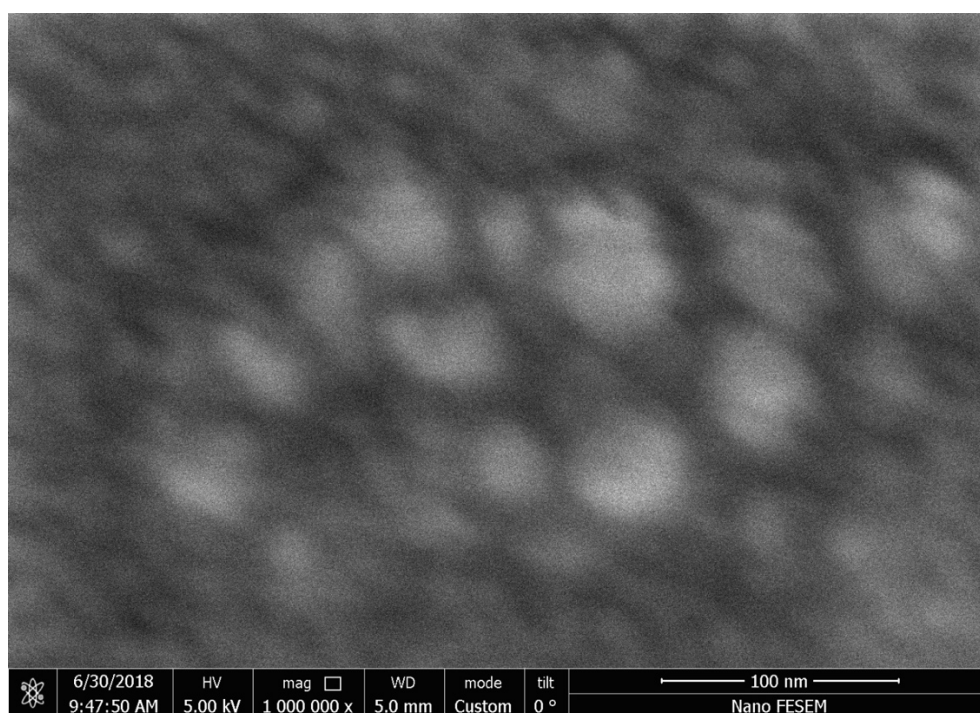


Figure 3. FESEM microscopic image of gold nanoparticles.

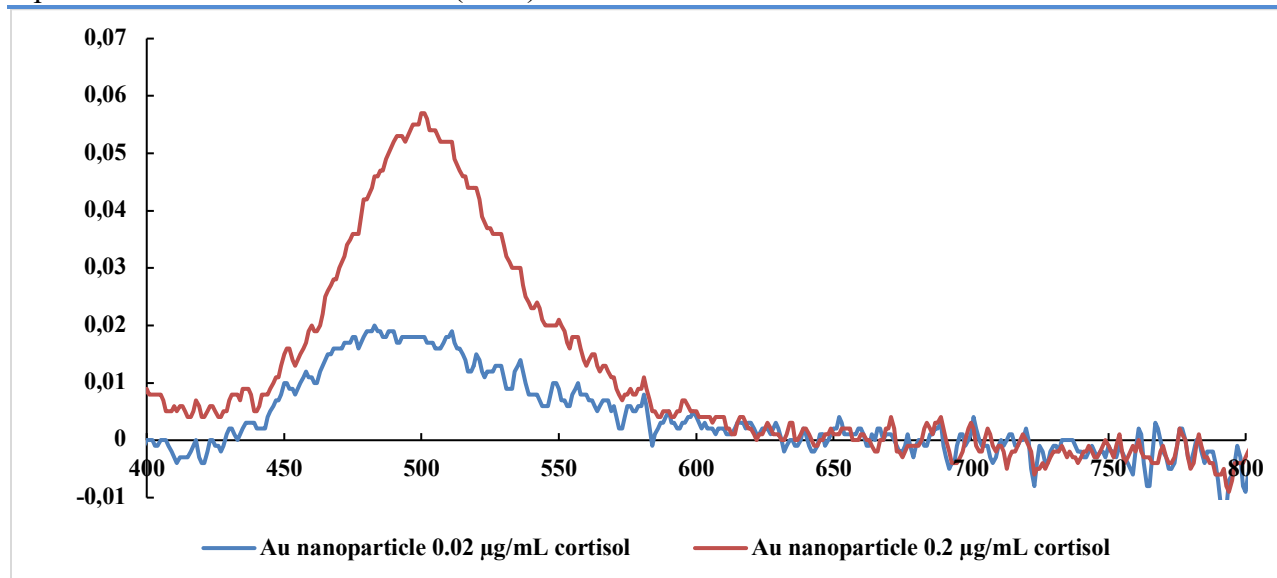


Figure 4. Absorbance spectra of aptamer functionalized with gold nanoparticle in the presence of different concentration of cortisol.

4. Conclusion

In summary, a novel colorimetric method for cortisol detection has been successfully constructed based on LSPR aptamer-selective sensing mechanism. Besides the common merits of colorimetric detection (low cost and simplicity and), the proposed method is fast and accurate quantification of cortisol. The detection limit toward the target is achieved as $0.02 \mu\text{g}\cdot\text{mL}^{-1}$.

Acknowledgments

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