Talanta 190 (2018) 140–146

Contents lists available at ScienceDirect

Talanta

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

A sensitive fluorometric DNA nanobiosensor based on a new fluorophore for tumor suppressor gene detection

Maryam Darestani-Farahani[a], Farnoush Faridbod[a,*], Mohammad Reza Ganjali[a,b]

[a] Center of Excellence in Electrochemistry, School of Chemistry, College of Science, University of Tehran, Tehran, Iran
[b] Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Nanobiosensor
DNA
Tumor suppressor
Gold nanoparticles
Fluorescence
Fluorophore

ABSTRACT

In this study, a sensitive fluorescent DNA nanobiosensor has been developed to determine DNA sequence of a well-known tumor suppressor gene, Adenomatous Polyposis Coli (APC). The design of the nanobiosensor was carried out using a synthetic organic ligand as a new fluorophore. The response mechanism of the nanobiosensor was based on DNA hybridization. The new fluorophore was assembled on gold nanoparticles (Au NPs) to enhance the sensitivity of the nanobiosensor response. The fabricated DNA nanobiosensor showed a fluorescence emission at 477 nm by exciting wavelength of 360 nm. By addition of the ssDNA target, the fluorescent emission of the nanobiosensor enhanced linearly in the range from 3.3×10^{-10} to 1.1×10^{-9} mol L^{-1} with detection limit of 1.3×10^{-11} mol L^{-1}. The proposed DNA nanobiosensor responded selectively to its complementary strand in comparison with non-complementary and three mismatched bases. The nanobiosensor had also a fast response time with acceptable repeatability. Finally, the performance of the DNA nanobiosensor in biological fluid, serum plasma, was investigated and a satisfactory results were obtained.

1. Introduction

Geneticists, for many years, have tried to find the reason of disease through detecting changes or mutations in genes. Study of human gene status can be a critical issue in diagnosis of many diseases such as diabetes, cardiovascular disease, autoimmune disorders, psychiatric illnesses and even cancer [1]. It is important in determining a successful treatment, early detection of tumors and improving the survival rates in the cancer infected patients [2]. Progress in DNA science and finding more about genes and their functions, make the analysis of genes at the molecular level possible. New technologies and analytical methods introduce day by day to find detection methods to be able to determine the changes in nucleic acids sequencing more sensitive and accurate [3].

In recent years, some methods have been used to determine the status of tumoric genes such as bisulphite genomic DNA sequencing, Southern blot, restriction enzyme-PCR, denaturing high-performance liquid chromatography (DHPLC) and electrochemical methods [4–8]. These methods need enzymes for specific modification of DNA bases [9,10] antibodies and proteins that bind to cytosine [11–14] or for some specific chemical changes [15,16] which are costly and time-consuming. In the other hand, some DNA biosensors have been fabricated recently that are known as a great alternative method due to their fast response, cost-effectiveness, being more sensitive and selective in addition to easy applications as a rapid test in decision making [17,18].

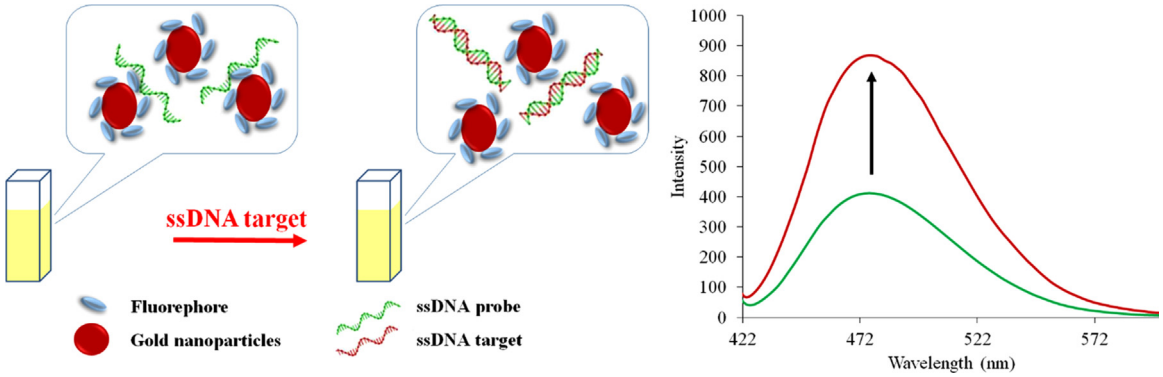
Currently, finding direct detection of DNA sequences without using chemical or enzymatic treatments is the area of interest for scientific communities and industries [19]. Designing a DNA biosensor based on fluorescent detection can be a good suggestion for its high sensitivity and high selectivity [20]. Also application of nanomaterials can improve the biosensors responses and may provide an inexpensive, rapid and easy to use analytical tools. Moreover, biosensors based nanomaterials are capable to be portable for point of care diagnosis [21].

To design a DNA biosensor, a synthetic organic ligand is used as a novel fluorescent DNA probe. It was selected from triazole thiol indole derivatives. In the other hand, selected ligand has interesting interaction with gold nanoparticles and has high photo-stability and quantum yield. The IUPAC name for the selected ligand is 4-((thiophen-2-yl) methyleneamino)-5-(1H-indol-3-yl)-4H-1,2,4-triazole-3-thiol. It is characterized by an intense $\pi \rightarrow \pi^*$ transition absorption bands at 288 nm and 334 nm and a fluorescence at 477 nm. The investigation of ligand interactions with DNA is important to understand the mechanism of interaction and design new ligands [22].

Adenomatous Polyposis Coli (APC) is known as a tumor suppressor genes. It can inhibit the uncontrolled growth of cells which possibly be responsible for cancerous tumors. APC can be related with some

* Corresponding author.
E-mail addresses: faridbodf@khayam.ut.ac.ir, faridbodf@ut.ac.ir (F. Faridbod).

https://doi.org/10.1016/j.talanta.2018.07.042
Received 5 January 2018; Received in revised form 11 July 2018; Accepted 12 July 2018



Scheme 1. Schematic representation of the designed DNA nanobiosensor for determination of APC gene sequence.

cellular routes, such as apoptosis, cell-cycle regulation, cell adhesion, cell migration, microtubule assembly and cell fate determination [23]. Mutations in the APC gene have been seen in about 80% of all human colon cancers. Thus, it can be used as a marker for early diagnosis of cancerous transformations [24]. Here, due to high importance of determining this gene, a specific sequence of APC gene previously reported [19] was selected as the target molecule.

In this research, we introduced a new fluorophore as a fluorescent probe in fabrication of a DNA biosensor that can determine the concentration of APC gene in human plasma samples. Fluorophore molecules are immobilized on the gold nanoparticle to improve the sensitivity and then ssDNA probe is added to the solution. The response mechanism of the nanobiosensor was based on DNA hybridization. As illustrated in Scheme 1, the addition of ssDNA target results in gradual increase of the emission. The fabricated optical DNA nanobiosensor has fast response, with good selectivity and sensitivity.

2. Experimental details

2.1. Instruments and measurements

All fluorescence experiments were recorded with a Perkin Elmer LS-45 fluorescence spectrometer (UK). Ultra violet- visible (UV–Vis) spectroscopy was performed using a Specord 250 spectrophotometer (Germany) and Infra-Red (IR) spectra were recorded using a Bruker-Tensor 27.

2.2. Materials

All chemicals used were purchased from Merck Co. (Germany). Tris-hydrochloric acid (Tris-HCl) and chloroauric acid ($HAuCl_4{\cdot}4H_2O$) were purchased from Sigma-Aldrich. Deionized water was used during the experiments.

The Organic ligands was synthesized in the medical science University of Tehran, Tehran, Iran. The tested 24 bp oligonucleotides for the study were purchased from Shanghai Generay Biotech Co. The base sequences are as follow:

Probe sequence (P): 5′-TCCGCTTCCCGACCCGCACTCCGC-3′;

Target sequence (complementary sequence) (T): 5′-GCGGAGTGCG GGTCGGGAAGCGGA-3′;

Three-base mismatched sequence (in CpG sites): 5′-GCCGAGTGCC GGTCGGGAAGCCGA-3′;

Three-base mismatched sequence (in non-CpG sites): 5′-GCGGTGT GCGGGACGGGATGCGGA-3′;

Non complementary sequence: 5′-CTTATCCTTTAGTTTATGTCT TAT-3′.

All oligonucleotides stock solutions were made with TE Buffer and kept in refrigerator before use. 0.01 mol L^{-1} Tris–HCl solution (in pH 8) and 0.001 mol L^{-1} EDTA solution were used to make a TE Buffer. Dilute solutions were prepared with 0.01 mol L^{-1} Tris-HCl buffer containing 0.5 mol L^{-1} NaCl in pH 7.4.

2.3. The synthesis of organic ligands

The general procedure for the preparation of the ligands was done through a well-known Schiff's Base reaction [25]. The general procedure was as follow: a mixture of a (0.01 mol) X compound (In ligand I, X is Thiophen-2-carbaldehyde; ligand II, Pyrrol-2-carbaldehye and ligand III, Furan-2-carbaldehyde) with 4-amino-5-(1H-indol-3-yl)-4H-1,2,4-triazole-3-thiol (0.01 mol) and a catalytic amount of acetic acid was refluxed for 5 h in absolute ethanol (20 mL). Then the solvent was evaporated to 5 mL, cooled to room temperature, and the product was

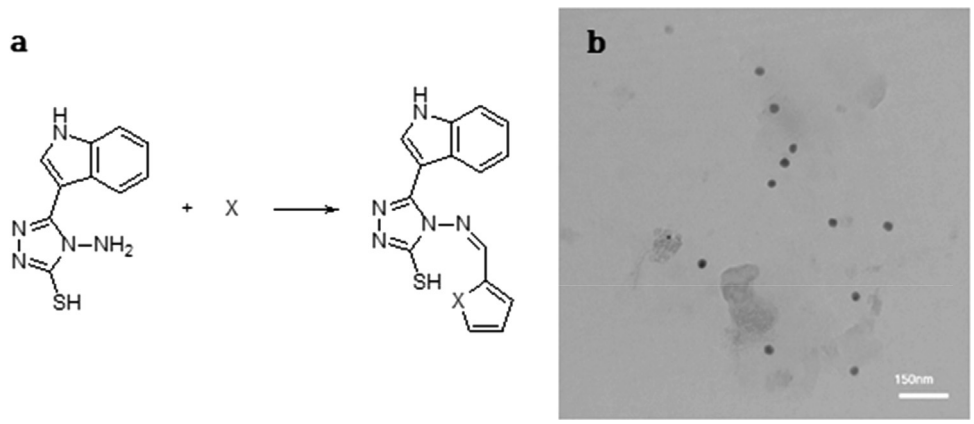


Fig. 1. (a) General synthesis of the ligand I, II and III (In ligand I, X is Thiophen-2-carbaldehyde; ligand II, Pyrrol-2-carbaldehye and ligand III, Furan-2-carbaldehyde) (b) TEM image of synthesized Au NPs.