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Synergistic effect of magnetite and gold nanoparticles onto the response of a label-free impedimetric hepatitis B virus DNA biosensor

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ABSTRACT

A magnetite and gold nanoparticle modified carbon paste electrode (CPE) was prepared for the immobilization of a thiol modified Hepatitis B virus (HBV) probe DNA and determination trace amount of target HBV DNA. Indeed, the sensing platform integrated two nanoparticles that had previously been employed individually in the DNA biosensors. The proposed DNA biosensor could measure target HBV DNA virus concentration with a low detection limit of 3.1 ($\pm$0.1) $\times$ 10–^{13} M, which was greatly lower than the detection limit reported with gold or magnetite nanoparticles alone. The change of interfacial charge transfer resistance (R_{CT}) was confirmed the hybrid formation between probe and target HBV DNA. The R_{CT} difference (before and after hybridization with the target HBV DNA) was in a linear relationship with the logarithm of complementary oligonucleotide concentrations in the range of 8.3 ($\pm$0.1) $\times$ 10^{-13} to 6.4 ($\pm$0.2) $\times$ 10^{-7} M. In addition, the novel methodology for specific DNA sequence detection was highly selective, repeatable, and reproducible. Finally, this work was successfully utilized for the sensitive and label free impedimetric determination of HBV target DNA in the urine and blood plasma samples.

1. Introduction

Viral hepatitis due to hepatitis B virus (HBV) is a major public health problem all over the world. Hepatitis B is a complex virus, which replicates primarily in the liver, causing inflammation and damage, but it can also be found in other infected organs. For this reason, the hepatitis B vaccine is strongly recommended for health-care workers, people who live with someone with hepatitis B, and others at higher risk. In many countries, hepatitis B vaccine is inoculated to all infants, and it is also recommended to previously unvaccinated adolescents [1,2].

The determination of specific DNA sequences provides the basis for detecting a wide variety of microbial and viral pathogens. Traditional methods for DNA sequencing, based on the coupling of electrophoretic separations and radio-isotopic (^{32}P) detection, are labor and time consuming. Electrochemical hybridization biosensors for the detection of DNA sequences may greatly reduce the assay time and simplify its protocol. Such fast on-site monitoring schemes are required for quick preventive action and early diagnosis [3].

Carbon paste electrodes (CPEs) are one of the most commonly used electrodes in the electrochemical investigations. The application of CPEs in analytical chemistry has attracted considerable attention in recent years [4–8].

Although the initial application of electricity to biological materials goes back to the first experiments of Luigi Galvani [9], it has been only recently that the coupling of electrochemistry and biology has resulted in a synergistic field of science, i.e., bioelectrochemistry. The field is growing rapidly and is beginning to have a significant impact on the practice of medicine [10], biology [11], pollution control, energy conversion, food technology, and other areas. Among the facets of bioelectrochemistry that are being investigated are mechanisms of electron transfer by enzymes and other macromolecules, electrochemically stimulated tissue and bone growth, electrical homeostasis, uses of biologically active molecules and organisms in combination with electrodes, and many more [12].

Electroanalytical chemistry can play a very important role in the protection of our environment [13]. In particular, electrochemical sensors and detectors are very attractive for on-site recording of priority pollutants, as well as for addressing other environmental needs. Such devices satisfy many of the requirements for on-site environmental analysis. They are inherently sensitive and selective towards electroactive species, fast and accurate, compact, portable and inexpensive. Such capabilities have already made a significant impact on decentralized clinical analysis [14].

In electrochemical DNA biosensors, detection is based on the variation in the electrical properties of the DNA modified electrode before and after hybridization, which may be due to the change of double-layer capacitance, electronic transfer resistance, impedance or current. In most of the DNA biosensors which produce either electrical or photonic signals, the probes are either modified by electrochemically active

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substances or labeled; which includes, organic dyes, metal nanoparticles, thiol-linked, enzyme labeled, Biotin labeled, fluorescent and radioactive labeled probes [15,16]. Although, these indirect (labeled) methods proved to be effective, they are complex, expensive, time-consuming, and also may cause DNA damage which in turn affects the DNA recognition. Thus, the direct or the label-free DNA biosensor has become extremely beneficial and can be readily employed for the real-time detection.

Electrochemical impedance spectroscopy (EIS) detection has enabled simplification of the detection and enhancement of the sensitivity of the measurement. EIS recently has been chosen as a main detection method because it has some important advantages over a number of electrochemical methods such as amperometry and potentiometry [12]. As it is known, EIS is an electrochemical technique that provides the examination of electrical properties of electrode surface and binding kinetics of molecules between electrolyte and electrode surface. Therefore, it can be used for biomolecular recognition, biomolecular bindings and biomolecular interactions between molecules such as DNA–DNA, DNA–protein, receptor–ligand, protein–ligand, antibody–antigen, and ion channels–ligands. As a consequence, EIS provides label-free detection without chemical transformation, and most of the bimolecular events can be monitored by EIS expeditiously [17–20].

Due to their small size (1–100 nm), nanoparticles exhibit novel material properties that differ considerably from those of the bulk solid-state [21]. Especially in recent years, the interests in nanometer-scale magnetic particles are growing [22,23]. However, as the particle size decreases, the reactivity of the particle increases and the magnetic properties are influenced more by surface effects [24].

Magnetic nanoparticles (MNP) are a valuable class of nanomaterials with unique properties that remain distinct from those that manifest their bulk counterparts. MNPs typically are in the size range from a few nanometers up to tens of nanometers, which places them at dimensions that are smaller or comparable to those of biological entities, such as cells and proteins. As the magnetic particle size descends from the micron scale to the 10–20 nm size range, the magnetic properties pass from the multi-domain state through higher co-reactivity single-domain regime to the super-paramagnetic regime [25].

Gold and iron oxide magnetic nanoparticles have attracted particular attention and are considered as excellent candidates for surface functionalization because they are easily synthesized, biocompatible, and have a greater surface area. Furthermore, the use of thiol chemistry on the gold surface facilitates attachment of functionalized molecules [25–28].

In this work, we used magnetic and gold nanoparticles (GNP) for enhancing the electrochemical signal of the DNA biosensor and increasing the sensitivity of the biosensor for HBV DNA detection. The use of 3-(trimethoxysilyl)-1-propanthiol (TMSPT) for the passivation of magnetic nanoparticles can increase the stability and reproducibility of the modified electrode. On the other hands, the free S-H group of TMSPT in the functionalized magnetic nanoparticles provides an enhanced loading interface for the gold nanoparticles and probe HBV ss-DNA. The proposed electrochemical DNA biosensor exhibited excellent sensitivity, good reproducibility and selectivity for determination trace amount of HBV DNA. These superior features can be related to unique synergism between magnetic and gold nanoparticles in the ss-DNA immobilization and also the high sensitivity of EIS technique. The promising performance of the developed DNA electrochemical biosensor makes this methodological study and application attractive in the HBV-DNA analysis.

2. Experimental

2.1. Materials

Potassium monohydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium ferrocyanide [$K_4Fe(CN)_6$], potassium ferricyanide [$K_3Fe(CN)_6$], $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, and ammonia solution were prepared from the Merck Company (Germany). All the DNA oligonucleotides used in this study were obtained from the Aminsan Company (Iran) with the following sequence:

HBV Probe (S1): S-H-5′- GAGGAGTTGGGGGAGCACATT-3′.

Complementary HBV DNA (S2): 5′-AATGTGCTCCCCCAACTCCTC-3′.
Three base mismatched DNA (S3): 5′-AGTGTGCTCTCCCAACTCCGC-3′.
Non-complementary DNA (S4): 5′-CACCTCAACCCCCTCGTGTAA-3′.

All oligonucleotides were dissolved in 0.1 M Phosphate buffer solutions (PBS) (pH 7.0) and were kept in the refrigerator at 4 °C. Phosphate buffer solutions (0.1 M) were prepared from KH_2PO_4 and K_2HPO_4. All other chemicals were of analytical reagent grade, and double distilled water (DDW) was used throughout.

2.2. Instruments

Electrochemical experiments, including cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) were carried out with an Autolab 302 N electrochemical workstation (Metrohm-The Netherlands). The three-electrode system was composed of a platinum electrode as auxiliary (Azar electrode, Iran), an Ag/AgCl (saturated KCl) as a reference electrode (Azar electrode, Iran), and an unmodified/modified carbon paste as working electrode. The morphologies of the synthesized and modified magnetic nanoparticles were characterized with a JEM 1200 EXII transmission electron microscope (JOEL company-USA). A Shimadzu XRD-6000 X-ray diffractometer (Japan) with Cu-Kα radiation (wavelength = 0.154 nm) was used for powder X-ray diffraction (XRD) measurement. The powder was packed in the glass sample holder and the XRD patterns were collected between $10° < 2\theta < 90°$ with dwell time of 2 s and a step size of 0.02°. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet Magna-IR 550 spectrometer (USA) in the region of 4000–400 cm^{-1} with 4 cm^{-1} resolution.

2.3. Preparation of bare and functionalized magnetic nanoparticles

The magnetic nanoparticles (MNP) were prepared according to our previous works [29,30]. Briefly, 11.68 g of $FeCl_3 \cdot 6H_2O$ and 4.3 g $FeCl_2 \cdot 4H_2O$ were dissolved in 200 mL deionized water under nitrogen atmosphere with a vigorous stirring at 80 °C. Then, 45 mL of aqueous ammonia solution (25%) was added, and the color of the solution turned from dark orange to black immediately. The precipitates were washed three times with deionized water and twice with 0.02 M sodium chloride. The prepared magnetite suspension was placed in 250 mL round flask and was allowed to settle. For preparation of TMSPT coated magnetic nanoparticles (MNP/TMSPT), the supernatant was removed, and 80 mL aqueous solution of TMSPT 10% v/v and 60 mL of glycerol were added, respectively. The pH of the suspension was adjusted to 4.6 using glacial acetic acid, and the mixture was then stirred at 90 °C for 2 h under a nitrogen atmosphere. After cooling to room temperature, the suspension was sequentially washed with deionized water, methanol, and deionized water. Finally, the MNP/TMSPT composite was stored in deionized water at a concentration of 40 g/L.

Gold nanoparticle modified MNP (MNP/GNP) or gold nanoparticle modified MNP/TMST (MNP/TMSPT/GNP) were prepared according to literature [31,32]. Briefly, 0.229 g of sodium citrate was dissolved in 100 mL of DDW, and the solution was heated to 99 °C under vigorous stirring. Then 1 mL of the prepared MNP or MNP/TMSPT suspension was added to the solution. Finally, 5 mL of 10 mM $HAuCl_4$ was slightly added to the solution, the water bath was removed, and the suspension was stirred for 15 min. The solid was removed again by a magnet and was rinsed with deionized water as the claret-red suspension was cooled down to the ambient temperature. Then, MNP/GNP or MNP/