DNA enrichment by functionalized magnetic nanoparticles for on-site and fast detection of virus in biomedical application

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Abstract. Label-free electrochemical DNA sensor is a promising technique for simple, fast, on-site virus detection. However, the low sensitivity is still a challenge for this method. This report shows a way to improve the sensitivity by magnetic enrichment of the DNA concentration before measuring DNA concentration by the DNA sensor. The enrichment was performed by conjugating superparamagnetic nanoparticles with the DNA probe single strand. Then, the system of nanoparticles-DNA probe was matched with the target DNA followed by magnetic decantation and compulsorily unfolding the DNA strands at a high temperature. The obtained solution was determined by the electrochemical DNA sensor. The results showed that the DNA enrichment process by magnetic nanoparticles improved the sensitivity of the electrochemical DNA sensor to about 200 times, which can be used for on site virus detection.

Keywords: DNA sensors, DNA amplification, label free detection.

1. Introduction

In recent years, many studies have been devoted to develop DNA sensors due to the simplicity, specificity, exceptional sensitivity and selectivity for the detection of specific genes [1–3]. Biosensors have opened a perspective to overcome most of disadvantages of the conventional detection methods for the applications in food, agriculture, environment, clinics and drugs... [3–12]. With the ability of amplification from small amounts of DNA into readable quantities, the conventional methods such as polymerase chain reaction (PCR) and real-time PCR have been applied mostly in the fields of biomedicine. However, these techniques require time, sample preparation, well-equipped apparatus and well-educated operators [3, 9].

DNA sensor is considered as a promising tool in pre-diagnostics, prevention and control of the infectious diseases which supposed to be able to be used for real-time and on site analysis [2, 3, 9]. High sensitive and selective DNA sensors are of great importance in diagnosis of genetic diseases, detection of infectious agents, and identification in forensic and environmental cases [1–12]. There are various types of DNA sensors which have been developed. Methods used for DNA detection in those sensors have been reported to be based on radiochemical, enzymatic, fluorescent, electrochemical, optical, and acoustic wave techniques [13]. Optical DNA sensors are used predominantly and promising results have been reported. The disadvantage of the optical sensors, however, is the requirement of a separate labeling process, equipment to stimulate the transducer, high complexity,
and thus, a higher cost in order to conduct an analysis [14].

Electrochemical method for hybridization detection is an alternative way compared to well-developed fluorescent detection. Over the past decade, a significant progress has been made towards the development of electrochemical DNA sensors. Considerable advantages of these devices are ascribed to their potential for obtaining specific information in a faster, simpler and inexpensive way. In addition, the sensors have a high potential for automation and miniaturization since only basic electrochemical equipment is required [2, 12, 15–17]. However, in practice the concentration of the DNA trace is too small to be detected. For in-lab analysis, samples can be pretreated before the final measurement. The pretreatment must be simple and useful. One of the pretreatments is magnetic enrichment. In this work, we reported the sample enrichment by using magnetic nanoparticles to detect low concentration Herpes virus sample.

Magnetic nanoparticles (MNPs) are the subject of many recent studies, especially in biological applications such as cell separation and sorting, magnetic drug delivery, magnetic resonance imaging [19] due to the fact that MNPs have following advantages: - small particle size of order of nm allow MNPs attach to biological objects without changing their functions; - large surface areas permit strong bond between MNPs and biological entities as well as drug molecules which connect to biological objects through MNPs; - strong magnetic nature of nanoparticles (NPs) allow control system of biological objects/MNP by an external magnetic field, which leads to changes in some physical or chemical properties of the system and the environment in an expected way. In addition, at the scale smaller than a critical length, ferromagnetic behavior disappears and changes to a superparamagnetic (SP) state, which shows strong magnetic properties under an applied field and nonmagnetic behavior when the field is removed.

Figure 1. Schematic illustration of the functionalization of magnetic nanoparticles.

The critical length in this case is of the order of a few tens of nm at which the thermal agitation dominates the ferromagnetic order of the particle and, as a result, the particle does not show magnetic properties under zero fields. SP state is very important in many biological applications because it prevents the aggregation in the bloodstream. Magnetite nanoparticles are the most commonly used magnetic materials in the field of biophysics due to its high saturation magnetization $M_s$ (90 Am$^2$/kg), biological compatibility, environmental stability, and technical simplicity and inexpensiveness of preparation process. Therefore, we used magnetite nanoparticles for study in this work.

2. Experiments

Magnetite nanoparticles have been prepared by the conventional coprecipitation of Fe$^{3+}$ and Fe$^{2+}$ salts by NH$_4$OH at room temperature. In a typical synthesis, 4.17 g of FeCl$_3$·6H$_2$O and 1.52 g of FeCl$_2$·4H$_2$O (such that Fe$^{3+}$/Fe$^{2+} = 2$) were dissolved in 80 ml water (concentration of Fe$^{2+}$ was 0.1 M) with vigorous stirring. A solution of 6 ml NH$_4$OH 35% was added with the rate of 1 drop per second at room temperature during constant stirring. Black precipitates of magnetite were formed and isolated from the solvent by magnetic decantation. Water washing and decantation processes were repeated four times to remove excess solution. Procedures are conducted under N$_2$ atmosphere. Nanoparticles with a diameter of about 15 nm have been obtained for further treatments.
The surface functionalization of MNPs was based on the reaction between amino group of the 3-aminopropyl triethoxysilane (APTES) and the phosphate group of DNA sequence. The functionalization procedure of MNPs is the following. Nanoparticles were mixed with a solution containing methanol and chloric acid (vol. ratio: 1/1) for 30 min. Then MNPs were immersed with another solution consisting of 30 vol. % of APTES and 70 vol. % of ethanol for one hour to obtain APTES attached to the Fe₃O₄ MNPs (APTES-Fe₃O₄). The APTES-Fe₃O₄ MNPs were reacted with 1-Methyllmidazole (MIA) activated DNA (MIA-DNA). The MIA-DNA was a product of the reaction between 5'-phosphorylated DNA and [3-(Dimethylamino) propyl]ethylcarbodiimide (EDC). The role of MIA was to stabilize the activated EDC molecules which were labile in aqueous solution. The oxygen atom in phosphate group of the DNA (5' terminal) in MIA-DNA was used as interface media to bind DNA probe with amino group of APTES (figure 1, left side). As a result, the surface of the Fe₃O₄ MNPs was coated with the DNA probe (DNA-Fe₃O₄). Schematic illustration of amino modified magnetite nanoparticles is presented in figure 1. The DNA-Fe₃O₄ was heated in de-ionized water at 37°C for 18 hours. DNA probe, that was the Herpes virus in this work, with a specific sequence for HSV-1 of 5'-AT CAC CGA CCC GGA GAG GGA C-3' and complementary DNA target sequence for HSV-1 of 3'-TA GTG GCT GGG CCT CTC CCT G-5' was supplied by Invitrogen Life Technologies Company.

As described in the previous work [21], the DNA concentration in real sample was very small. With the current sensor configuration, real concentration is sometimes out of detection range. This is why the samples need to be enriched before the analysis. After that, the probe DNA attached to MNPs hybridized with the target DNA by stirring. In a typical experiment, 1 ml of probe DNA attached to the Fe₃O₄ nanoparticles was mixed with 1 ml HSV1-DNA sequence for 15 minutes at room temperature. For faster hybridization process, the reaction cell was stabilized at 37°C. Magnetic decantation was applied to obtain the condensed MNP/DNA system. The condensed sample was heated up to 98°C for DNA unfolding in 15 minutes and rapidly cooled down to room temperature. The probe DNA attached to the Fe₃O₄ nanoparticles were removed from the sample by applying a magnetic field from outside. The enriched sample was finally analyzed by electrochemical DNA sensor.

The electrochemical sensor to measure concentration of DNA consisted of a pair of microelectrodes placed on the surface of a silicon substrate. One microelectrode served as the working electrode, another as the reference. The dimensions of the electrode were 30×70 μm² (figure 2). The signal measurement relied on the hybridizing of two DNA sequences which changes electric density on the surface of the DNA sensor. The reference signal of an AC current with frequency of 10 KHz and amplitude of 100 mV was given by a Lock-in Amplifier (SR830). The output signal was acquired by measuring the voltage change between two 1 KΩ resistances. The change of the voltage resulted from the hybridization possibility of the probe DNA attached on the surface and the target DNA strands between the two electrode. If there was no hybridization, there would be no change of the output voltage.
The possibility of hybridization depended on the concentration of the target DNA. Therefore, the output signal was a function of the concentration of the target DNA strands. Our study revealed a linear dependence of the output signal and the concentration in the range from 10 nM/l to 10 μM/l. We used the DNA sensor to determine the concentration of DNA of Herpes virus before and after the DNA magnetic enrichment. Some available tools allow calculating the molecular weight of DNA sequence just simply by the number of bases of the DNA. In our case, the molecular weight of 21 base HSV1 sequence is 6.4 K.Daltons. Limit of the sensitivity of the sensor is 10 nM/l. For samples with concentration lower than the limit, other techniques must be applied. To overcome this difficulty, we combined magnetic enrichment with the electrochemical sensor. A certain volume of solution of 0.1 nM/l of the Herpes DNA was magnetically concentrated from 20 - 200 times using MNPs and an external magnetic field of a strong commercial magnet. After magnetic decantation, the concentration of the DNA increased to the value higher than the sensitivity of the sensor. The concentration of the concentrated solution obtained from measurement by electrochemical sensor was compared to the value obtained from the reduction of the solution volume before and after enrichment process.

Transmission electron microscope measurements were carried out using a Transmission Electron Microscope (TEM) JEM-1010 working at an accelerating voltage of 100 kV. Samples for TEM were prepared by ultrasonically dispersing the products into absolute ethanol, placing a drop of this suspension onto a copper grid coated with an amorphous carbon film or onto a copper plate, respectively, and then drying in vacuum. Fourier Transformed Infrared (FTIR) Spectra were recorded in the transmission mode on a Nicolet Impact 410 spectrometer. Magnetic properties were measured by Vibrating Sample Magnetometer DMS 880-CTS.

3. Results and discussion

The transmission electron microscope (TEM) image of the ATPES coated Fe₃O₄ nanoparticles was given in figure 3. Particles were spherical with diameters from 13 - 18 nm. The particles were separated due to a layer of ATPES coating on the surface of the particles. As illustrated in figure 1, APTES was bound to the surface of nanoparticles leaving the NH₂ group outwards which can conjugate with phosphate groups of DNA probe. Magnetic measurement showed a SP behavior with the saturation magnetization of 65 emu/g.

Fourier Transformed Infrared Spectrum of the DNA-APTS complexes (figure 4) revealed absorption bands at 2972 and 2925 cm⁻¹ of the stretching vibration of the C-H bond, 1091 cm⁻¹ of the stretching vibration of the C-N bond, 1051 cm⁻¹ of the stretching vibration of the Si-O bond, 885 cm⁻¹ of the bending vibration of the NH₂ group, 1750-1600 cm⁻¹ of the vibration plane of the G-C pairs and A-T base pairs, 1085 cm⁻¹ of the backbone phosphate.
AC reference signal (10 KHz, 100 mV sine wave) generated by the Lock-in Amplifier SR830 was applied on the two microelectrodes of the DNA sensor. The output signal was acquired by measuring the voltage change between the two 1 K resistances. The probe DNA with concentration of 100 mM/l was immobilized on APTES coated electrodes by a way which was similar to the way the probe DNA attached to the MNPs. All measurements were performed at room temperature. In this experiment, five DNA sensors were used to test the hybridization of DNA sequences.

Figure 4 presents the dependence of the output signal on the volume of the solution containing 0.1 nM/l of the Herpes DNA before the magnetic enrichment. The initial solution contained 0.1 nM/l of the DNA which was much smaller than the sensitivity of the sensor. Therefore, the measurement of the solution before magnetic enrichment was almost zero (figure 5, opened square). After magnetic enrichment, depending on the initial volume of the solution, the output signals linearly increased with the increasing of the solution volume: the higher the volume, the higher the concentration. As a result, the higher output signals were obtained. This means that the concentration of HSV-1 DNA was much condensed after the enrichment. The linearity of the output signals is very important for the calibration. The UV-VIS spectrometer (Ultrospec 2000) was also used in this work to determine the concentration of DNA target after the enrichment. The concentration obtained by comparing the initial volume and the final volume was almost consistent with the concentration obtained from the electrochemical sensor. With the highest initial volume that was used in our studies, the concentration after magnetic enrichment was 200 times higher than the initial concentration. With the magnetic enrichment process, we can measure solution with the low concentration of 0.1 nM/l, which is 10 times smaller than the limit of the electrochemical sensor.

Figure 4. FTIR spectra of the probe DNA attached to the nanoparticles.

Figure 5. Dependence of the output signal on the volume of the solution containing 0.1 nM/l of the Herpes DNA before the magnetic enrichment.
4. Conclusion
This work reported the application of Fe$_3$O$_4$ nanoparticles for DNA enrichment for onsite and fast label-free electrochemical DNA sensor. The magnetic enrichment was simple which can be applied easily in under equipped laboratories. It is promising for on-site analysis of viruses in remote areas.

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