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Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 341–345

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

Detection of hepatitis B virus by piezoelectric biosensor

Xiaodong Zhou [a], Lijia Liu [a], Min Hu [b], Leilei Wang [a], Jiming Hu [a,*]

[a] *Department of Analysis-Measurement Science, Wuhan University, Wuhan 430072, PR China*
[b] *Faculty of Life Science, Wuhan University, Wuhan 430072, PR China*

Received 16 May 2001; received in revised form 27 May 2001; accepted 30 May 2001

Abstract

A highly sensitive piezoelectric HBV DNA biosensor has been developed based on the sensitive mass-transducing function of the quartz crystal microbalance and the speciality of nucleic acid hybridization reaction. HBV nucleic acid probe was immobilized onto the gold electrodes of a 9 MHz AT-cut piezoelectric quartz crystal with the polyethyleneimine adhesion, glutaraldehyde cross-linking (PEI-Glu) method or the physical adsorption method. The coated crystal with the PEI-Glu method to immobilized HBV nucleic acid probe showed the better results than the physical adsorption method with respect to sensitivity reproducibility and stability. The frequency shifts of hybridization have better linear relationship with the amount of HBV DNA, when the amount was in range 0.02–0.14 μg/ml. The crystal could be regenerated nearly five times without perceptible decrease of sensitivity.

Keywords: Hepatitis B virus; Nucleic acid hybridization; Quartz crystal microbalance

1. Introduction

The hepatitis B is a familiar disease in China, and it is a contagious disease induced by the virus of the hepatitis (HBV) DNA. It is particularly important to detect the concentration of virus nucleic acid in human body. The determination can provide the key foundation for the diagnosis and remedy. Many traditional immunoassays are widely used [1,2], but suffer from disadvantages including complex, time consuming procedures and potentially hazardous or expensive materials.

Increasing attention is being paid to develop the piezoelectric nucleic acid biosensor, especially those that can be used to study various DNA hybridization interactions between complementary strands [3] and real-time detection of DNA binding process [4], of the intercalation of dye molecules into DNA [5], and the enzymatic cleavage of nucleic acid [6]. A sensor consists of an oscillator circuit and thin slice of AT-cut piezoelectric quartz and produce an alternating electric field, which drives the quartz crystal to oscillate at a characteristic constant frequency,

* Corresponding author. Tel.: +86-27-8768-2439; fax: +86-27-2788-2136.
E-mail address: jmhu@whu.edu.cn (J. Hu).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00538-6

determined by the crystal mass. The adsorption on the surface of crystal increases the rigid mass of crystal, and causes the crystal to change its oscillation frequency according to the Sauerbrary equation [7], which can then be used to precisely quantify with a detection sensitivity in the ng range.

On the present study, we develop a novel piezoelectric HBV DNA biosensor for the detection of HBV DNA. The HBV nucleic acid probe is immobilized onto the gold electrode with polyethyleneimine adhesion, glutaraldehyde cross-linking (PEI-Glu) method and physical adsorption method. This sensor represents rapid, sensitive and reliable alternation in the determination of HBV DNA.

2. Exprimental

2.1. Apparatus and materials

The piezoelectric crystals used in this study were gold deposited AT-cut with a 9 MHz basic resonance frequency from Beijing Chengguang Co. (Beijing, China). The crystal consisted of a 12.5×0.2 mm (diameter, thickness) quartz wafer, placed between 6 mm gold electrodes. The oscillator circuit was constructed from a transistor–transistor circuit (TTL-IC). The frequency was monitored with a high-resolution frequency counter (CN3165, Sampo Co., Taiwan, China). The crystals were washed in an ultrasonic cleaner (B2000, Branson Co., Shanghai, China). Thermostat bath (CS501-3 Chongqing Instrument Co., Chongqing, China) was used to stabilize the temperature.

Polyethyleneimine and glutaraldehyde were purchased from Wuhan Medical and Chemical Reagent Company (Wuhan, China). HBV DNA and the hepatitis virus nuclear acid probe were obtained from Guangzhou Biological Produce Company (Guangzhou, China). All other reagents and solvents were analytical reagent grade. Deionized ultrapure water (DW) was used.

2.2. HBV nucleic acid probe immobilization procedures

The crystals used were immersed in 1.2 mol/l NaOH for 10 min, washed with DW, then dipped in 1.2 mol/l HCl for 5 min and washed with DW. The crystals were subsequently washed with DW and ethanol in a ultrasonic cleaner and dried in air.

After the crystal had been cleaned and activated, the following two methods were employed for the immobilization of HBV nucleic acid probe:

2.2.1. Immobilization with PEI-Glu method

A 5 μl polyethyleneimine (40 g/l) were dripped onto the surface of gold electrodes of crystal, then distributing uniformly and dried in air at room temperature. Washed with DW and ethanol, the crystal dried in air at room temperature. Put 10 μl glutaraldehyde (0.3 mol/l) on the thin layer of polyethyleneimine. After dried for 40 min at the room temperature, the crystal were washed with DW and dried in air, then determine the resonance frequency (F_1). Suitable cubage virus nucleic acid probe was dropped on the thin layer of PEI-Glu membrane. After dried for 40 min at the room temperature, the crystal were rinsed with PBS (pH 7.2) and DW and dried in air, then the resonance frequency (F_2) were measured.

2.2.2. Immobilization with physical adsorption

The crystals were activated by placing two drops of concentrated HCl on the gold electrodes for 2 min, and washed with DW. Then, the resonance frequency (F_1) was determined. A suitable cubage virus nucleic acid probe was put on the electrode surface by microinjector. After dried at the room temperature for 2 h, the crystal were subsequently rinsed with PBS (pH 7.2) and DW, dried in air, then the resonance frequency (F_2) was measured.

2.3. Measurement procedures

A suitable amount virus DNA was dropped on the surface of gold electrodes, which had a thin layer of virus probe, then the crystal were placed

at 85 °C for 10 min and re-annealed rapidly on the ice for 5 min, and hybridized at 25 °C for 40 min. The crystal were washed well with PBS (pH 7.2) and DW, dried in air and the new frequencies (F_3) were measured. The frequency changes ($\Delta F = F_3 - F_2$) are related to the amounts of virus DNA to hybrid with the virus nucleic acid probe to immobilize on the crystals.

2.4. Regeneration of crystals

The crystals used were immersed in 1.2 mol/l NaOH for 10 min, washed with DW, then dipped in 1.2 mol/l HCl for 5 min and washed with DW. The crystals were subsequently washed with ethanol and DW in an ultrasonic cleaner.

3. Results and discussion

3.1. Comparison of the immobilization procedures

The two different immobilization methods are compared in Table 1. The PEI-Glu method gave the better results in terms of sensitivity, reproducibility and stability. Although physical adsorption method was much simpler, but it took too much time and the amount of nucleic acid probe to immobilize on the crystal could not repeat. The experiment result shows that the PEI-Glu method can make a quite fixed film and provide more binding sites for the immobilized nucleic acid probe, and the property does not affected by the change of pH, solvent or temperature.

Table 1
Characterization of two immobilization procedures

Immobilization method	ΔF_1 (Hz)	ΔF_2 (Hz)	ΔF_3 (Hz)
PEI-Glu	268	250	248
Physical adsorption	125	103	158

The frequency changes of coating with virus nucleic acid probe on the electrode three times are compared.

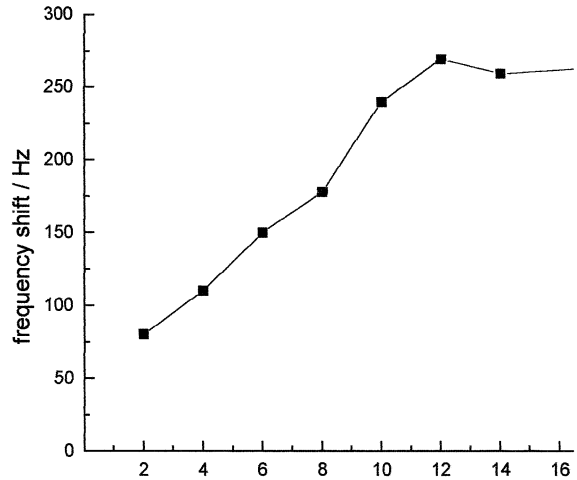

Fig. 1. Relationship between amount of virus probe and frequency shift.

3.2. Effect of the amount and time of HBV nucleic acid probe to immobilize on the crystal

The amount of virus nuclei acid probe to immobilize on the crystal and the immobilization time were the major factors to influence the binding capacity of the probe on the PEI-Glu membrane. The crystals had sufficient adsorption in 12 μl solution, at higher amount, the frequency change due to virus probe adsorption was no longer dependent on the probe amount (Fig. 1). The adsorption was rapid in the first 30 min, then it increased slowly, reaching equilibrium after 40 min. So, we used 40 min to immobilize the virus nucleic acid probe.

3.3. Effect of hybridization reaction time

The correlation between the hybridization reaction time in range of 10–60 min and frequency shifts are showed in Fig. 2. It seems that the frequency shifts increase with the increase of the hybridization reaction time in range of 10–30 min. Beyond the time of 40 min, the frequency shifts did not increase with a further increase of reaction time. Obviously, the hybridization reaction had generally finished at 40 min, so the hybridization reaction time 40 min was adopted for the following experiments.

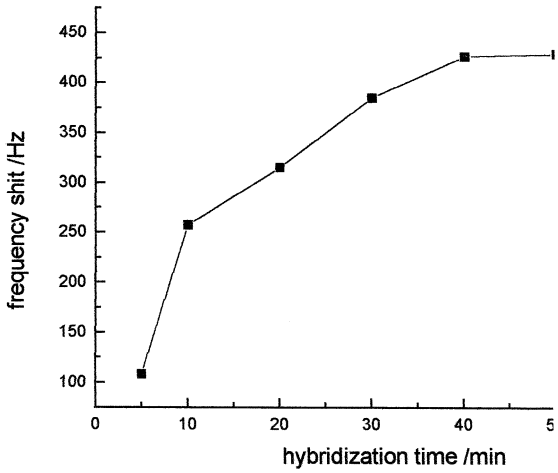

Fig. 2. Relationship between hybridization time and frequency shift.

3.4. Main characteristic

Fig. 3 shows frequency shifts and the correlation between the HBV DNA concentration in range of 0.01–0.20 μg/ml and frequency change resulting from binding of virus at the optimum conditions. The frequency shifts of hybridization have better linear relationship with the amount of HBV DNA, when the amount was in range 0.02–0.14 μg/ml. Above the amount, the frequency changes were no longer dependent on the concentration of HBV DNA.

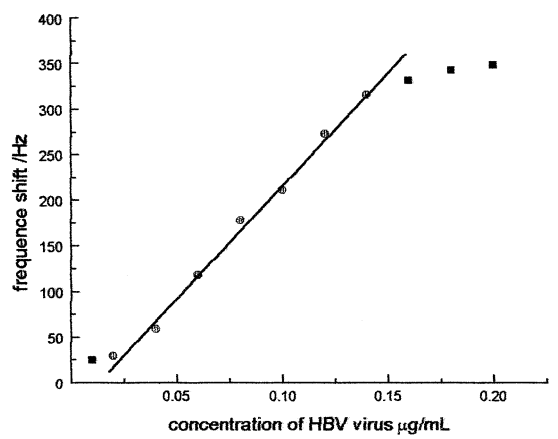

Fig. 3. Correlation between concentration of HBV DNA and frequency shift.

Table 2
Typical detection sensitivity after regeneration of the crystal

Number of use	1	2	3	4	5	6	7
F_3–F_2	220	227	225	218	210	198	156

The frequency changes of coating with virus nucleic acid on the electrode seven times are compared.

The selectivity of the sensor was evaluated by dropping the solutions of oligo$(dA)_{20}$, oligo$(dT)_{20}$, oligo$(dG)_{20}$, and oligo$(dC)_{20}$ 0.11, 0.55, 0.11, and 0.11 μg/ml, respectively. The measurement steps were exactly as described previously for HBV DNA. The frequency changes are below 20 Hz, and has little influence to the measurement of HBV DNA. These results suggested that these nucleic acid did not in interfere significantly with the detection.

3.5. Regeneration of the crystals

The regeneration of the crystal used is a crucial part to develop the practical sensor. In this study, we employed a new dissociation method for regeneration of the crystals used as described in the experiment part. Using this procedure, the strong acid and strung alkali solutions could lead nucleic acid on the crystal surface to inactivate and desorb, then the washing in a ultrasonic cleaner could dissociate the adsorbed materials. The crystal could be regenerated nearly five times without decreasing of detectable activity, but there after the sensitivity of the sensor declined sharply (Table 2).

With other methods, such as 0.1 mol/l glycine–HCl buffer solution (pH 2.3), 0.1 mol/l phosphate saline–citria buffer solution (pH 2.4), 0.2 mol/l ethanolamine (pH 8), 4 mol/l KCNS and 8 mol/l urea [8], the hybridized product of nuclei acid could not usually be desorbed completely. At the same time, these method involved relatively aggressive chemicals that could reduced five time and reusability of the coated crystal.

4. Conclusion

The results demonstrate that it is available to detect the HBV DNA with the piezoelectric HBV biosensor. The PEI-Glu method for the immobilization of HBV nucleic acid probe is a stable and sensitive alternative. The sensor was successfully used for the detection of HBV DNA in the concentration range of 0.02–0.14 μg/ml. The crystal could be reused five times by a regeneration procedure. The sensitivity, the selectivity and reusability of this sensor promise a good future for further application.

Acknowledgements

This work was support by the grant of National Natural Science Foundation of China 39670218.

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