Biosensors and Bioelectronics 60 (2014) 259–264

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A new system for the amplification of biological signals: RecA and complimentary single strand DNA probes on a leaky surface acoustic wave biosensor

Liqun Zhang [a,1], Yunxia Wang [a,1], Ming Chen [b], Yang Luo [a], Kun Deng [a], Dong Chen [a], Weiling Fu [a,*]

[a] Department of Laboratory Medicine, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China
[b] Department of Laboratory Medicine, Daping Hospital, Third Military Medical University, Chongqing 400042, PR China

ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form
10 April 2014
Accepted 21 April 2014
Available online 28 April 2014

Keywords:
Leaky surface acoustic wave (LSAW)
Peptide nucleic acid (PNA)
Sensor
RecA protein
Signal amplification

ABSTRACT

This research describes a new amplification signals system of the leaky surface acoustic wave (LSAW) bis-peptide nucleic acid (bis-PNA) biosensor for the simple, sensitive and rapid detection of the target double-stranded DNA (dsDNA). The system consists of a RecA protein-coated complementary single-stranded DNA (cssDNA) probe complex that amplifies the biological signal to improve the sensitivity of the biosensor. The bis-PNA probe for detecting HPV was first immobilized on a gold surface membrane of the detection channel. After the probe was completely hybridized with the corresponding target DNA, different concentrations of the "RecA protein-complementary single strand DNA probe" were added to react with the bis-PNA/dsDNA complex. The phase shift of the LSAW biosensors, which was measured and found to be most significant when the RecA protein was 45 μg/mL and the ATPγS was 2.5 mmol/L. Compared with other concentrations ($P<0.01$) of RecA and ATPγS, the value of the phase shift was (11.74 ± 1.03) degrees and the ratio of the phase shift and hybridization time clearly outperformed that of the other concentrations. Compared to the direct hybridization of the bis-PNA probe and the target DNA sequence, the sensitivity was effectively improved and the detection time was significantly shortened. PNA binding adjacent to the area of the target sequence homologous to the probe significantly increased the yield of the hybridization reaction between the PNA/dsDNA complex and the RecA protein-coated cssDNA probe. In this condition, the phase shift was significantly obvious and the detection time was significantly shortened. In conclusion, the combination of the RecA protein-coated cssDNA probe and the LSAW bis-PNA biosensor provides sensitivity and simple and rapid detection of clinical trace pathogenic microorganisms.

1. Introduction

Leaky surface acoustic wave (LSAW) biosensors are a new technology, which have remarkably high base frequencies (from 100 MHz to 2 GHz) and higher sensitivities than traditional quartz crystal microbalances (QCM) (Yao et al., 2009; Xu et al., 2012). The detection principle of a LSAW biosensor uses the special cutting of piezoelectric crystals, such as cutting a 36° rotation in the *Y*-direction and propagating $LiTa0_3$ in the *X*-direction, to activate a LSAW. Tiny changes in the loading quality of the LSAW propagation path (e.g., the nucleic acid reactions occurring between the target sequences and the fixed probes) cause changes in the surface boundary conditions of the crystals, resulting in a corresponding change in the phase and frequency of LSAW propagation. As the change in the phase and frequency of the signal from the output transducer of the biosensor is extracted, the test target sequences that react with the fixed probes on the crystal surface of the biosensor can be detected with high sensitivity. The corresponding relation is $\Delta f=-(K_1+K_2)f_0^2\Delta m/A$, with K_1 and K_2 being the constants of the dielectric material, f_0 being the center frequency of the LSAW (GHz), A being the region area of the quality attached, Δm being the quality attached, and Δf being the change of the corresponding frequency. Measuring the change in frequency or phase allows the detection of tiny changes in the quality attached to the crystal surface. At present, most DNA sensors can detect single-strand target DNA but not dsDNA, and the target sequences must undergo PCR amplification before they can be detected using biosensors. This greatly limits the possible applications of biosensor technology (Tombelli et al., 2000;

* Corresponding author. Tel.: +86 23 68754429; fax: +86 23 65460909.
E-mail addresses: zlqtmmu@gmail.com (L. Zhang), weilingfu@yahoo.com (W. Fu).
[1] These authors contributed equally to the study.

http://dx.doi.org/10.1016/j.bios.2014.04.037
0956-5663/

Zhou et al., 2001; Mannelli et al., 2003). However, PNA can bind to homopurine targets in dsDNA via strand invasion, forming a stable $(PNA)_2$/DNA triplex (Nielsen et al., 1991; Egholm et al., 1993, 1995; Bentin et al., 2006).

In a previous study, we developed a LSAW bis-PNA biosensor platform and successfully quantified genomic dsDNA with a detection limit of 1 pg/L (Wang et al., 2009). Although the detection of trace pathogenic microorganisms has been achieved, previous studies found that the phase change in the pg-level detection of pathogenic microorganisms is small, only approximately 0.8° (Wang et al., 2009). Signal amplification strategies must be applied to improve the sensitivity and detection of trace pathogenic microorganisms (Ali and Li, 2009). Sensitive and selective DNA detection has become increasingly important in the rapid detection of trace pathogens for diagnosis (Sun et al., 2010). Currently, various labeling methods have been reported for signal amplification of biosensors, such as immobilization technology, nano-materials, enzyme-biocatalyzed deposition, and so on (Zhong et al., 2010). Although these methods have many advantages, including high sensitivity, they have several major drawbacks. These complex procedures long analysis cycles, insufficient sensitivity or specificity, and indirect hybridization with dsDNA at room temperature. Compared with these techniques, a RecA protein-coated cssDNA probe is simple to operate and offers high specificity for the detection of dsDNA.

RecA is an *Escherichia coli* protein essential for the repair and maintenance of DNA and plays an important role in recombination-based DNA repair in bacteria (Cox et al., 2000; Kowalczykowski, 2000; Lusetti and Cox, 2002; Drees et al., 2004; De Vlaminck et al., 2012). RecA binds strongly to single-stranded DNA (ssDNA) to form a nucleoprotein filament, which can bind to homologous or partially homologous sequences within dsDNA targets (Cox, 2007; Roy et al., 2009). The RecA protein has more than one DNA binding site and can thus hold a single strand or double strand together. This feature makes it possible to catalyze a DNA synapsis reaction between a DNA double helix and a homologous region of single stranded DNA. The role of PNA facilitation in the reaction between RecA protein cssDNA probes and the dsDNA has already been established (Belotserkovskii and Zarling, 2002).

The purpose of this research is to evaluate a highly effective method of rapidly detecting dsDNA micro-pathogenic microorganisms using a RecA protein-coated cssDNA probe complex as a new biological signal amplification system based on the LSAW bis-PNA biosensor. To investigate our biosensor system, we have chosen the dsDNA virus Human Papillomavirus (HPV) as the target pathogenic microorganism. Based on the full-length sequence of the human HPV genome, we designed and synthesized three different ssDNA probes: T1, T2, and T3. First, the bis-PNA probe for detecting HPV was immobilized on the gold membrane surface of the detection channel. After the probe was completely hybridized with the corresponding target DNA, different concentrations of the RecA protein cssDNA probe were added to react with the bis-PNA/dsDNA complex. RecA requires ATPγS for all these activities and hydrolysis during a strand exchange reaction (Yamazaki et al., 2003). The concentrations of RecA protein were optimized, and the phase shift and reaction time were observed under optimum conditions.

2. Materials and methods

2.1. Reagents

Bis-PNA probes with the sequence 5′-SH–$(CH_2)_6$–$(Lys)_3$–TTTTCTTCCT–$(egl)_3$–TCCTTCTTTT–Lys (in which egl denotes the linker, SH denotes a thiol group, and Lys denotes lysine) were purchased from Chengdu CP Biochem Co., Ltd. (Chengdu, China). The probe was purified first with reversed-phase HPLC and characterized using MALDI-TOF spectrometry. Adenosine 5′-O-(3-thiotriphosphate) (ATPγS) was obtained from Sigma (St. Louis, USA). BSA was purchased from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). RecA protein was purchased from BioLabs (New England Biolabs, USA). HPV Genotyping test kits were purchased from Hybribio Biotech Co., Ltd. (Guangdong, China). The ssDNA targeting HPV genes, purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China), were characterized as follows: T1 is adjacent to the bis-PNA-binding site by 6 bp and consists of the sequences 5′-AA TAG ATG GAG TTA ATC ATC and 5′-GAT GAT TAA CTC CAT CTA TT; T2 is adjacent to the bis-PNA-binding site and consists of the sequences 5′-CGA TGA AAT AGA TGG AGT TA and 5′-TAA CTC CAT CTA TTT CAT CG; and T3 has a 3 bp overlap with the PNA binding site and consists of the sequences 5′-AAA CGA TGA AAT AGA TGG AG and 5′-CTC CAT CTA TTT CAT CGT TT. The pBR322-HPV 18 plasmid was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The plasmid extraction kit was purchased from BioDev-Tech. Co., Ltd. (Beijing, China).

2.2. Apparatus

The LSAW biosensor was obtained from the 26th Research Institute at the Chinese Electronic Scientific and Technical Group Company (Chongqing, China). The phase shift data were displayed on the main display screen and could be read directly by a computer connected to a network analyzer (R3767CG, ADVANTEST, Japan). The order was sent automatically to the network analyzer, and the detection data were simultaneously acquired from the LSAW biosensor and recorded on the computer through a GPIB controlling card (Agilent Co., USA). The detecting platform of the LSAW biosensor is shown in Fig. 1A. Phase shift data collection, storage, and analysis were processed using self-developed biosensor monitor system software (BSMS 2.0). Hybribio rapid hybridization apparatuses were purchased from Hybribio Biotech Co., Ltd. (Guangdong, China).

2.3. Extraction of HPV genomic DNA

The purified full-length plasmid DNA of HPV 18 was used as a standard for the method development. Hind III was used as a marker. Molecular grade pure water without DNA extract was used as a negative control.

2.4. Detection procedure

The bis-PNA (2.0 μmol/L) was immobilized on the gold electrode surface through the thiol method by adding 25 μL of 20 mmol/L PBS buffer with pH 6.6 to the detection and reference (negative control) channels (Wang et al., 2009). The phase (P_0) was monitored until a steady baseline was obtained at 55 °C. Then, 25 μL of 100 μg/L target genomic DNA was added to the detection and reference channels and hybridized with the bis-PNA probe at 55 °C. When the reaction ended, the other steady-state phase was taken as P_1. The phase shifts were calculated using the equation $\Delta P = P_1 - P_0$. The equilibrium time for hybridization was also recorded.

2.5. PNA hybridization between the RecA protein-coated cssDNA probe and dsDNA targets

2.5.1. Effects of RecA protein concentration on hybridization

To obtain RecA protein-coated cssDNA probes, 2.5 μm of ssDNA fragments in 14 μL were denatured from the dsDNA by incubating in a boiling water bath for 3 min and then chilled in ice for 2 min.

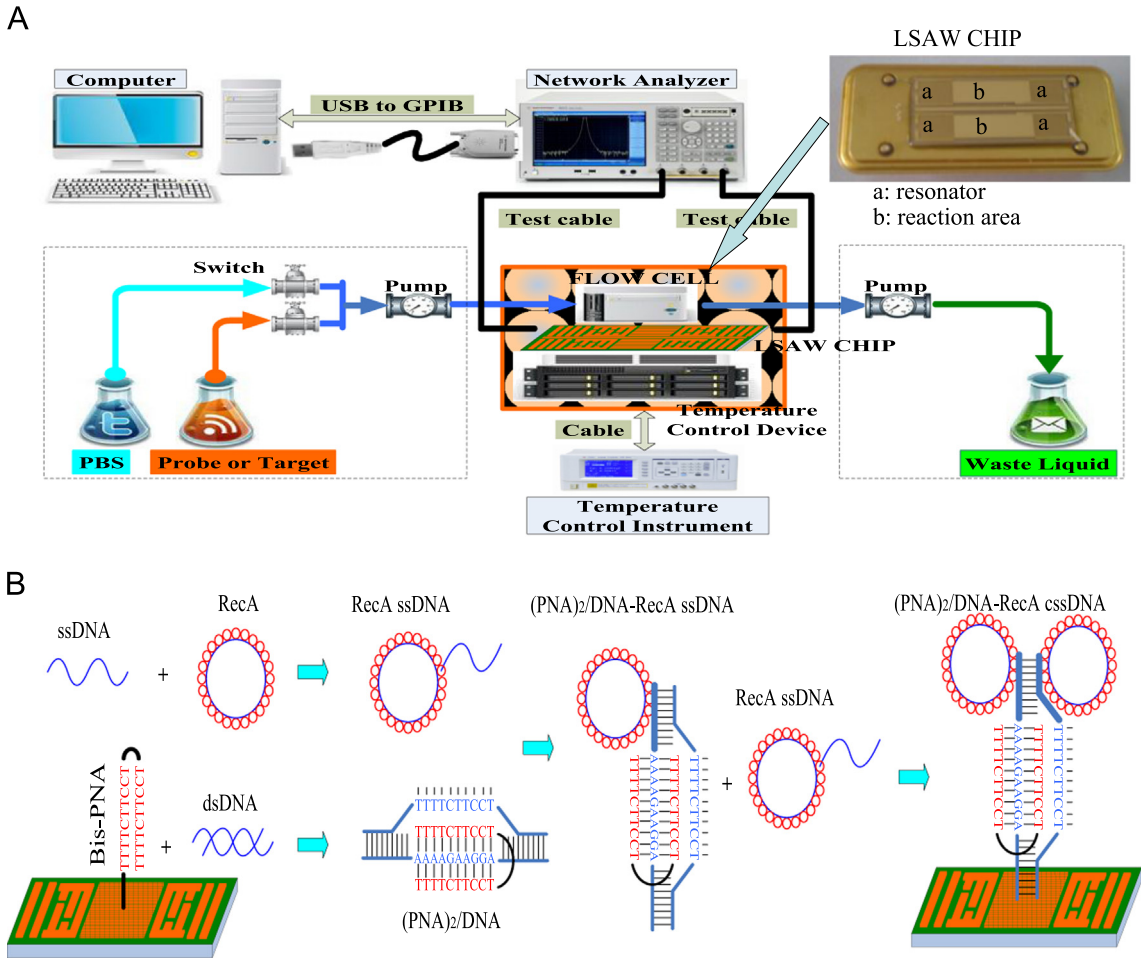

Fig. 1. Schematic diagram of (A) the detecting platform provided by the LSAW biosensor and (B) the cssDNA probes hybridized to a target DNA molecule. The detection process is shown.

Next, 14 μL of a freshly prepared mixture containing 7.0 μL of buffer (50 mm Tris acetate (pH 7.5), 250 mm sodium acetate (pH 7.0), 10 mM magnesium acetate, and 5 mM DTT) and 3.5 μL of 2.5 mm ATPγS was added, immediately followed by 0.5 μL of different concentrations of RecA protein (15, 30, 45, 60, and 75 μg/mL). The mixture was incubated for 17 min at 37 °C. After stabilization at 55 °C, 40 μL of 100 μg/L HPV genomic DNA was added to the detection cell. Bis-PNA and the target DNA were incubated in 100 μL of hybridization buffer for 10 min at room temperature. Next, 24.8 μL of RecA-coated cssDNA at different concentrations was added to the biosensor, and the hybridization reaction was incubated for 1 h at room temperature. PBS buffer was added to the biosensor as a negative control. The phase shift (ΔP) and the reaction time were recorded. The average value was calculated based on the data obtained by repeating the experiment 6 times. The cssDNA probes hybridized to the target DNA molecule is shown in Fig. 1B.

2.5.2. *Effects of ATPγS concentration on hybridization*

After the biosensor was equilibrated at room temperature, 40 μL of 100 μg/L HPV genomic DNA and 0.5 μL of 45 μg/mL RecA protein-coated cssDNA were added to the detection biosensor for hybridization with the bis-PNA probes. The total volume of the mixture was 100 μL. Then, 3.5 μL of 0.5, 1.5, 2.5, 3.5, and 4.5 mmol/L concentration of ATPγS was added to the biosensor. Triple-distilled water was added as a negative control in each trial. The phase shift (ΔP) and the reaction time were recorded, and the average value was calculated based on the data obtained by repeating the experiment 6 times.

2.6. *Clinical sample detection*

The study was approved by the local ethics committee, and participants signed an informed consent form. Clinical samples (subjects' secretions) were obtained from patients undergoing routinely HPV screening and from healthy volunteers at Southwest Hospital (Chongqing, China). The DNA extraction was performed using a DaAn Gene DNA kit (DaAn Gene Co., Ltd. Guangzhou, China) according to the manufacturer's protocol. Bis-PNA probe (2.0 μmol/L) was immobilized first on the detection channel. Then, 25 μL of 1.21 pg/L HPV DNA solution was extracted from the subject's secretions and added to the detection channel and reference channel for hybridization with the bis-PNA probe.

2.7. *Statistical analysis*

All experiments were performed at least 6 times. Data were analyzed with SPSS 13.0 statistical software. Phase shift data were presented as the mean values ± standard deviation. One-way ANOVA was used to determine the statistical significance of the differences between RecA protein concentration groups. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. PNA hybrid formation between target DNA and RecA protein-coated cssDNA probes

Bis-PNA exhibiting high affinity and high sequence specificity in binding to dsDNA may also recognize duplex homopurine sequences of DNA by strand invasion. When the dsDNA is in a negative supercoiled structure, it can instantly turn into a single chain in which a duplex is opened. The PNA strand can invade the DNA double helix and form a stable PNA–DNA–PNA triplex by Watson–Crick base pairing with the DNA strand. PNA binding interferes with the close contact of the displaced target strand with two other strands and with RecA protein, thus facilitating the "open conformation" of the complex. The PNA binding site is localized to the flank of the probe–target complex, and PNA binding provides stable unwinding at the flank, which further facilitates the open conformation. In this conformation, RecA protein can more easily penetrate the major groove inside the double helix DNA molecule and a complex can be formed with the first RecA protein-coated cssDNA strand. The displaced target strand is closely localized to the other strands and can interact with the complex. This process requires ATPγS hydrolysis to supply energy (Tang et al., 2000; Belotserkovskii and Zarling, 2002; Forget and Kowalczykowski, 2012). The binding between RecA protein and dsDNA in vitro did not need any facilitation from other proteins. RecA protein can rapidly promote the association of homologous DNA molecules, promoting the binding of the target strand of the dsDNA molecules with one molecular chain, resulting in heteroduplex DNA molecules with a PD-Loop formation. Thus, a stable complex was formed more easily by binding the $(PNA)_2$/DNA triplex to the RecA protein-coated cssDNA.

Signal amplification technology is key to improving the sensitivity of the biosensor and has great importance for detecting the small signal of the biological response. Direct detection of genomic dsDNA is the most prominent advantage of this method. In this study, the characteristics of the detection signal showed major changes in response to small changes in the mass of the biosensor surface. First, bis-PNA probe was immobilized on the surface of biosensor. Second, the bis-PNA probe was hybridized completely with the corresponding target DNA. Third, the RecA protein-coated cssDNA probe was added to react with the bis-PNA/dsDNA complex, which effectively increased the mass load of the biosensor surface and enhanced the detection signal, thus improving the sensitivity of the biosensor. In addition to improve the sensitivity of detection, this method reduces the detection time and successfully avoids PCR amplification. Rapid trace detection is also the highlight of this research: trace detection by this method can be completed within 30 min, and the whole detection process, including DNA extraction, RecA protein-coated cssDNA probe signal amplification, and real-time detection, can be done within 2 h. Thus, this method allows the early detection of pathogenic microorganisms and provides a basis for the early diagnosis of infectious diseases.

3.2. Amplification and specificity of RecA protein reaction

The amplification efficiency and specificity of the RecA protein reaction plays a very important role in analyzing biological samples. To evaluate the amplification efficiency and specificity of the biosensor in liquid phase detection, we compared the phase shifts caused by HPV genomic DNA. In our proposed method, we employed RecA protein to enhance the sensitivity of detection. The RecA protein-coated cssDNA probe can increase the mass loading. In brief, after the biosensor was equilibrated in PBS buffer, the RecA protein-coated ssDNA modification was added to samples of a negative control (PBS), BSA solution, HBV genomic DNA, HPV genomic DNA (1.21 pg/L), and HPV genomic DNA. Because HPV probes will not hybridize with BSA and HBV, the phase shift of the biosensor rapidly reaches equilibrium (Fig. 2, curves b and c). Conversely, the addition of 1.21 pg/L target DNA extracted from clinical specimens, the sensor's phase shift was approximately 0.8° (Fig. 2, curve d). After the addition of the "RecA protein-complementary single-strand DNA" probe, the phase shift was approximately 3.3° (Fig. 2, curve e), and the relative LSAW phase signal achieved a magnification of 4 times that of direct hybridization between the bis-PNA probe and the target DNA. Signal amplification resulted from the increased mass loading on the biosensor chip surface by RecA protein-coated cssDNA. No phase shift (ΔP) was observed in the BSA solution (negative control) or HBV dsDNA.

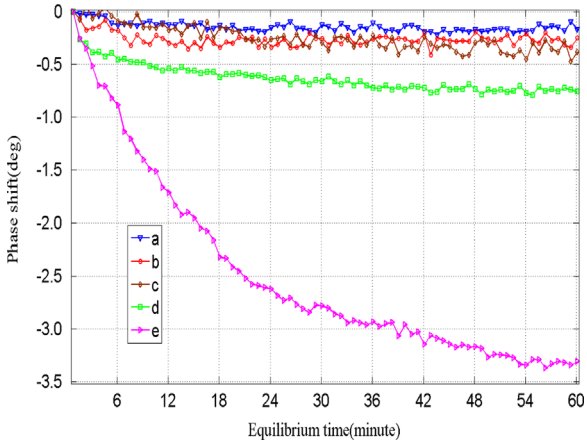

Fig. 2. Graphs of HPV detection in the clinical samples using the LSAW biosensor. From top to bottom: negative control (curve a), BSA solution (curve b), HBV dsDNA (curve c), HPV genomic DNA (1.21 pg/L) from clinical samples (curve d), and HPV genomic DNA with RecA protein-coated ssDNA modification (curve e). Error bars indicate the standard deviation ($n=6$), $^{**}p<0.01$.

We verified the results of the LSAW biosensor detection using the newly introduced, commercially available HPV Genotyping test kit for assaying the HPV genotype, which makes use of both DNA amplification and HybriBio's proprietary flow-through hybridization technique to simultaneously identify 21 HPV genotypes, including HPV 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, and Cp8034. Each of these was detected in the clinical specimens. The biotin control and internal control (IC) spots should be positive in detection. The test consists of a nylon membrane onto which HPV genotype-specific oligonucleotides probes have been immobilized. The results of this test are shown in Fig. S1. Because HPV probes will not hybridize with PBS, BSA, and HBV, the results of the HPV genotyping assay are negative (Fig. S1c–e). Conversely, following addition of the HPV DNA of clinical specimens, the results were positive for HPV 18 (Fig. S1b). These results confirmed that the LSAW biosensor was highly selective for HPV and exhibited strong resistance to interference from BSA and HBV.

3.3. Effects of RecA protein

The concentration of RecA protein plays a very important role in the hybridization reaction with the RecA protein-coated cssDNA probe. Fig. 3 displays the results obtained from different concentrations of RecA protein. It is obvious that the phase shift (ΔP) first increased and then decreased when the RecA protein concentration varied from 15 to 75 μg/mL (Fig. 3Aa). The phase shift (11.74°) was significantly higher when the concentration of RecA protein was 45 μg/mL compared with other concentrations ($p<0.01$).

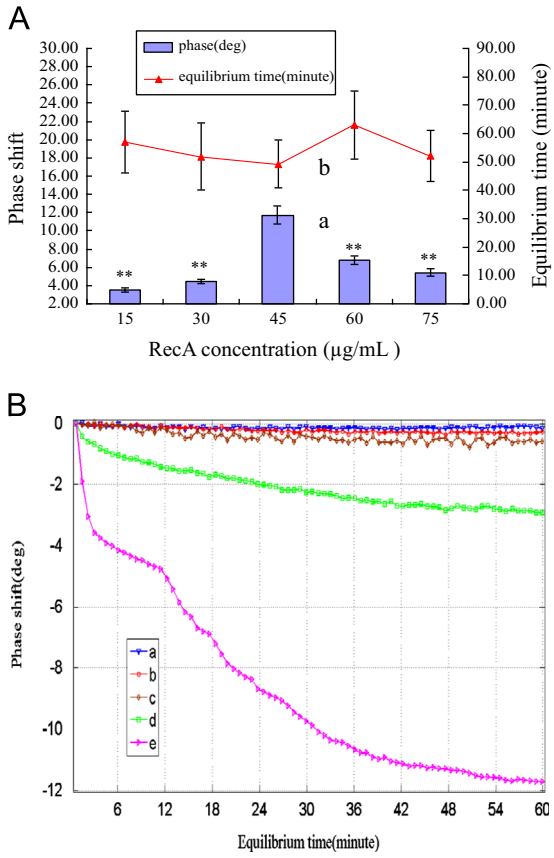

Fig. 3. Effect of RecA protein-coated cssDNA probe (A) concentrations for (a) the phase shift and (b) equilibrium time vs. RecA (45 μg/mL) group and (B) detection of HPV plasmid DNA. From top to bottom: the negative control (curve a), BSA solution (curve b), 100 μg/L HBV dsDNA (curve c), 100 μg/L plasmid DNA of HPV (curve d), and plasmid HPV genomic DNA of RecA protein-coated ssDNA modification signal (curve e). Error bars indicate the standard deviation ($n=6$), $^{**}p<0.01$.

This indicates that the concentration of 45 μg/mL was the best for the hybridization reaction. The high concentration of RecA protein resulted in a decrease in the phase shift of hybridization due to the positive charges existing in the RecA protein. When added to high concentrations of RecA, an excessively high concentration of positive charges can stabilize the dsDNA structure, resulting in an opening of the double helix of target DNA that is otherwise inaccessible to PNA. The time to equilibrium was similar at all concentrations ($p>0.05$) (Fig. 3Ab). Therefore, 45 μg/mL of RecA protein was selected as the optimal concentration of the amplification reaction reagent in the following experiments. At the best concentration of RecA protein, the ratio of the phase shift and the time spent on hybridization obviously outperformed that of the other concentrations (see Fig. S2). As is shown in Fig. 3B, the phase basically remains the same before and after addition of 100 μg of BSA and HBV dsDNA for hybridization (Fig. 3B, curves b and c). After the addition of 100 μg of HPV dsNDA, the phase shift was approximately 2.9° (Fig. 3B, curve d). After the addition of "RecA protein-complementary single-strand DNA" probes, the phase shift was approximately 11.8° (Fig. 3B, curve e). Compared to the direct hybridization of the bis-PNA probe and the DNA target sequence, the biosensor's response phase increased approximately 4 times and the biosensor's detection sensitivity increased significantly.

3.4. Optimization of ATPγS

The formation of complexes between nucleic acids and protein filaments by aggregation of RecA on ssDNA is a process that requires energy from the hydrolysis of ATPγS. This simultaneously promotes ATPγS connected complexes of nucleic acids and protein filaments and the further combination of two cssDNA with their corresponding target sequences. ATPγS enables the stretching of the nucleic acid–protein filament complex. Indeed, without ATPγS, these areas would be in a compressed state. Thus, the concentration of ATPγS is a critical factor, as it can affect the combination of the RecA protein-coated cssDNA and its corresponding target sequence. The reaction concentration of ATPγS was explored and optimized to determine the optimal effects of the RecA protein. Fig. 4a shows the effect of different concentrations of ATPγS on the LSAW phase shift. In this experiment, increasing concentrations of ATPγS duration (0.5, 1.5, 2.5, 3.5, and 4.5 mmol/L) were tested for their effects on signal amplification. Some interesting phenomena were observed in the hybridization experiments. The phase shift (ΔP) increased gradually as the concentration of ATPγS increased from 0.5 μm to 2.5 mmol/L. The maximum phase shift (ΔP) was obtained at approximately 2.5 mmol/L and then remained stable, indicating that saturation was achieved. When the concentration of ATPγS was higher than 2.5 mmol/L, the shift phase decreased. This may be attributed to the fact that when the concentration of ATPγS is higher than 2.5 mmol/L, the binding of RecA protein to DNA is inhibited by the increased amount of ATPγS. The time to equilibrium was similarly from 0.25 mmol/L to 4.5 mmol/L ($p>0.05$) (Fig. 4b). Thus, we selected 2.5 mmol/L ATPγS as the optimal concentration used for signal amplification in the hybridization process using the combination of RecA protein cssDNA and the target DNA (Fig. 4).

3.5. Effect of three different ssDNA probe locations

Routine hybridization conditions are completely unsuitable for RecA hybridization with dsDNA (Chen et al., 2008; Danilowicz et al., 2012). Because the ssDNA probe plays a very important role in hybridization between RecA and dsDNA, possible ssDNA probe locations were explored. Based on our preliminary experiments, the suitable range of locations for the ssDNA probe in promoting hybridization between RecA and dsDNA was quite narrow. In this experiment, we aimed to overcome this limitation by using three different probes that we added to the reaction system at three different locations. T1 is separated from the bis-PNA binding site by 6 bp, T2 is adjacent to the bis-PNA binding site, and T3 has a 3 bp overlap with the bis-PNA binding site (see Fig. S3).We recorded the phase shift and the hybridization equilibrium time using the biosensor (see Fig. S4). Our study results showed that the T2 probe provided the maximum phase shift (11.63°), which is significantly higher ($p<0.01$) than that of the other two probes (Fig. 5a).

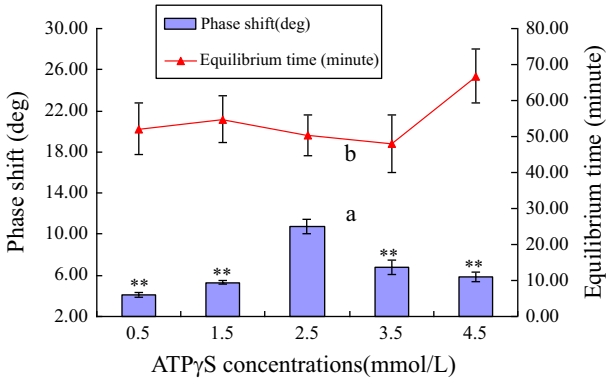

Fig. 4. Effect of different concentrations of ATPγS on (a) phase shift and (b) equilibrium time vs. ATPγS (2.5 mmol/L) group. Error bars indicate the standard deviation ($n=6$), $^{**}p<0.01$.

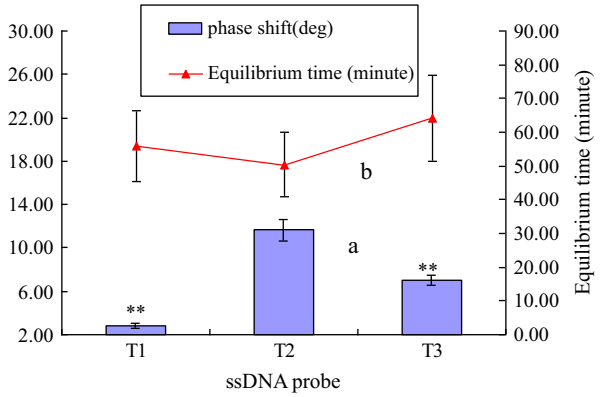

Fig. 5. Effect of the three different ssDNA probes for (a) the phase shift and (b) equilibrium time vs. T2 group. Error bars indicate the standard deviation ($n=6$), $^{**}p<0.01$.

The change in time to equilibrium (Fig. 5b) showed no obvious difference between all three probe locations ($p>0.05$). PNA binding adjacent to the area homologous to the probe target sequence significantly increases the yield of the hybridization reaction between the PNA/dsDNA complex and the RecA protein-coated cssDNA probe. Under these conditions, the quality of the biosensor loading is markedly increased and the detection signal of the biosensor is enhanced. To improve the RecA-mediated targeting reaction, the bis-PNA binding must occur adjacent to the area homologous to the probe (T2).

3.6. Sensitivity of RecA protein-coated cssDNA probes with LSAW biosensor

To validate the biosensor's sensitivity in liquid phase detection, we carried out a sensitivity assay. Under optimal experimental conditions, modified biosensors were used to detect various concentrations of plasmid HPV DNA. The results showed that within 1 pg/L–100 μg/L HPV, the logarithm of HPV concentration was linearly correlated with the phase shift. The linear regression equation for HPV DNA is $y=1.2492x+9.2528$, and the correlation coefficient is 0.9917. (see Fig. S5). In addition, as is shown in Figs. 2 and 3B, the curves reflecting the biosensor's detection of negative control (curve a), BSA (curve b) and HBV dsDNA (curve c) were basically the same, which approximated a straight line. Therefore, it is impossible to perform a sensitivity assay of BSA and HBV dsDNA.

4. Conclusions

In this study, we assessed a new technique based on a LSAW bis-PNA biosensor using signal amplification by a RecA protein-coated cssDNA probe to detect HPV genomic DNA. Using the LSAW bis-PNA RecA protein biosensor described here, we can amplify a signal with high sensitivity under optimized conditions without using expensive instruments. The hybridization reaction, the RecA protein-coated cssDNA probe signal amplification, and the LSAW detection can be achieved in the detection cell inside the biosensor, minimizing the risk of contamination. The workflow is simple and rapid, and the technique has a high sensitivity for the detection of trace gene dsDNA without amplification by PCR. Using the RecA protein-coated cssDNA probe, we have observed that the phase shift signal increased by 4 times. HPV genomic DNA at a level as low as 1.21 pg/L was detected, meaning that the detection signal was amplified in this assay. This new technique could be a useful supplement to clinical assay methods for the rapid identification of trace pathogenic bacteria.

Acknowledgments

The authors thank Professor Yun Zhang (English Department, Third Military Medical University, PR China) for a critical reading of the manuscript and kindly giving precious advice. This study was supported financially by the National Natural Science Foundation of China (81370049), the Application Development Projects of Chong Qing CSTC (cstc 2014yykf B10004), the Transformation of Scientific and Technological Achievements of the Third Military Medical University (2012XZH02), and the National Mega Projects for Key Infectious (2012ZX10004801-003-006).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.04.037.

References

Ali, M.M., Li, Y., 2009. Angew. Chem. Int. Ed. Engl. 48, 3512–3515.
Belotserkovskii, B.P., Zarling, D.A., 2002. Biochemistry 41, 3686–3692.
Bentin, T., Hansen, G.I., Nielsen, P.E., 2006. Nucleic Acids Res. 34, 5790–5799.
Chen, Z., Yang, H., Pavletich, N.P., 2008. Nature 453, 489–494.
Cox, M.M., 2007. Nat. Rev. Mol. Cell Biol. 8, 127–138.
Cox, M.M., Goodman, M.F., Kreuzer, K.N., Sherratt, D.J., Sandler, S.J., Marians, K.J., 2000. Nature 404, 37–41.
Danilowicz, C., Feinstein, E., Conover, A., Coljee, V.W., Vlassakis, J., Chan, Y.L., Bishop, D.K., Prentiss, M., 2012. Nucleic Acids Res. 40, 1717–1727.
De Vlaminck, I., van, L.M.T., Zweifel, L., den Blanken, J., Hooning, K., Hage, S., Kerssemakers, J., Dekker, C., 2012. Mol. Cell 46, 616–624.
Drees, J.C., Lusetti, S.L., Chitteni-Pattu, S., Inman, R.B., Cox, M.M., 2004. Mol. Cell 15, 789–798.
Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S.M., Driver, D.A., Berg, R.H., Kim, S.K., Norden, B., Nielsen, P.E., 1993. Nature 365, 566–568.
Egholm, M., Christensen, L., Dueholm, K.L., Buchardt, O., Coull, J., Nielsen, P.E., 1995. Nucleic Acids Res. 23, 217–222.
Forget, A.L., Kowalczykowski, S.C., 2012. Nature 482, 423–427.
Kowalczykowski, S.C., 2000. Trends Biochem. Sci. 25, 156–165.
Lusetti, S.L., Cox, M.M., 2002. Annu. Rev. Biochem. 71, 71–100.
Mannelli, I., Minunni, M., Tombelli, S., Mascini, M., 2003. Biosens. Bioelectron. 18, 129–140.
Nielsen, P.E., Egholm, M., Berg, R.H., Buchardt, O., 1991. Science 254, 1497–1500.
Roy, R., Kozlov, A.G., Lohman, T.M., Ha, T., 2009. Nature 461, 1092–1097.
Sun, C., Zhang, L., Jiang, J., Shen, G., Yu, R., 2010. Biosens. Bioelectron. 25, 2483–2489.
Tang, M., Pham, P., Shen, X., Taylor, J.S., O'Donnell, M., Woodgate, R., Goodman, M.F., 2000. Nature 404, 1014–1018.
Tombelli, S., Mascini, M., Braccini, L., Anichini, M., Turner, A.P., 2000. Biosens. Bioelectron. 15, 363–370.
Wang, Y., Chen, M., Zhang, L., Ding, Y., Luo, Y., Xu, Q., Shi, J., Cao, L., Fu, W., 2009. Biosens. Bioelectron. 24, 3455–3460.
Xu, Q., Chang, K., Lu, W., Chen, W., Ding, Y., Jia, S., Zhang, K., Li, F., Shi, J., Cao, L., Deng, S., Chen, M., 2012. Biosens. Bioelectron. 33, 274–278.
Yamazaki, J., Horii, T., Sekiguchi, M., Takahashi, M., 2003. J. Mol. Biol. 329, 363–370.
Yao, C., Qi, Y., Zhao, Y., Xiang, Y., Chen, Q., Fu, W., 2009. Biosens. Bioelectron. 24, 2499–2503.
Zhong, Z., Wu, W., Wang, D., Wang, D., Shan, J., Qing, Y., Zhang, Z., 2010. Biosens. Bioelectron. 25, 2379–2383.
Zhou, X.C., Huang, L.Q., Li, S.F., 2001. Biosens. Bioelectron. 16, 85–95.