แอฟฟินิตีไบโอเซนเซอร์แบบไม่ติดฉลากสำหรับไวรัสตัวแดงดวงขาว

Label-free Affinity Biosensor for White Spot Syndrome Virus

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บทคัดย่อ

คณะผู้วิจัยทำการผลิตโปรตีน WBP นำมาใช้เป็นวัสดุชีวภาพเพื่อพัฒนาแอฟฟินิตีไบโอเซนเซอร์แบบไม่ติดฉลากสำหรับตรวจหาปริมาณไวรัสตัวแดงดวงขาว ทำการตรึงโปรตีน WBP โดยผ่านเซล์ฟ-แอสเซมเบิลโมโนเลเยอร์บนอิเล็คโทรดทอง นำไปวิเคราะห์กับไวรัสตัวแดงดวงขาวโดยใช้ระบบไหลผ่าน เมื่อมีการจับกันของโปรตีนและไวรัส ตรวจวัดค่าความต้านทานรวมของวงจรไฟฟ้ากระแสสลับที่เปลี่ยนแปลงไป (ΔZ'') เมื่อทดสอบประสิทธิภาพของระบบไบโอเซนเซอร์โดยสร้างกราฟมาตรฐาน พบว่าสามารถตรวจวัดไวรัสตัวแดงดวงขาวในช่วงความเข้มข้น $1.6x10^{1}$-$1.6x10^{5}$ copies ซึ่งไบโอเซนเซอร์ที่พัฒนาขึ้นสามารถใช้งานได้ง่าย มีความน่าเชื่อถือ สามารถนำไปใช้ซ้ำได้ และอาจสามารถนำไปประยุกต์ใช้งานในอุตสาหกรรมการเลี้ยงกุ้งได้ต่อไป

ABSTRACT

We produced White Spot Syndrome Binding protein (WBP) and developed label-free affinity biosensor for the quantitative detection of WSSV in shrimp pond water. A WBP was immobilized on gold electrode through a self-assembled monolayer to detect WSSV under a flow injection system. Binding between WSSV and immobilized biological molecules was detected by an impedance change (ΔZ'') in real time. The sensitivity of biosensor was in the linear range of $1.6x10^{1}$-$1.6x10^{5}$ copies of WSSV. The system was highly sensitive for analysis of WSSV. The biosensor is simple to operate, reliable, reproducible and may be applied in areas of shrimp cultivation.

คำสำคัญ: ไวรัสตัวแดงดวงขาว, แอฟฟินิตีไบโอเซนเซอร์, อิมมูโนเซนเซอร์, กุ้ง, ความต้านทานรวมของวงจรไฟฟ้า

Keywords: white spot syndrome virus (WSSV), affinity biosensor, immunosensor, shrimp, impedance

INTRODUCTION

White spot syndrome virus (WSSV) is a major pathogen affecting the shrimp industry worldwide. It has caused economic losses to the shrimp farming industry and 100% mortality over a 3–7 day period. The infection may be caused by direct infection of the breeder or indirectly by crustaceans and water (Durand *et al*., 2003). However the water is a major pathway of WSSV entering the cultivation. Therefore, a sensitive, specific and rapid method to detect WSSV in water would be very useful for the detection of WSSV contamination before stocking ponds to prevent and control the spread of viruses which cause damaged to the shrimp farming (Samanman *et al*., 2011). Diagnostic methods for WSSV detection such as in situ hybridization, immunological methods and the polymerase chain reaction (PCR) have been developed. Although these molecular methods are sensitive and reliable, they are not feasible for detecting virus in pond-sites. Moreover, these methods are quite expensive and require many steps as well as an expert operator.

Therefore, label-free affinity biosensors which makes used of specific interaction between proteins are another alternative used to detect the virus. Due to the very specific, low cost of analysis, portable and can be reused (Caruso *et al*., 1997); a biosensor for detection WSSV in the shrimp pond water would be developed. The system base on flow injection technique using the White Spot Syndrome Binding protein (WBP) which can bound to VP26 of WSSV (Youtong *et al*., 2011) as a biological molecules immobilized on gold electrode via self-assembled monolayer. The binding between the WBP and WSSV were determined by electrochemical impedance to detect the change on the event.

MATERIALS AND METHODS

1. Material

WSSV stock solutions were provided by the Center for Genomics and Bioinformatics Research, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The viral titer ($1.6x10^{10}$copies) was determined by real-time PCR. Thioctic acid and 1-dodecanethiol (98%) were obtained from Sigma-Aldrich (Milwaukee, USA). N-(3-dimethylaminopropyl) - N-ethylcarbodiimide hydrochloride (EDC) and Nhydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals are an analytical grade. All buffers were prepared with deionized water treated with a reverse osmosis-deionized system (Millipore, Bedford, USA). Before use, buffers were filtered through a nylon membrane filter (Albet, Spain, pore size 0.20 μm) with subsequent degassing.

2. Production of WBP

The procedure was followed by Youtong *et al*., 2011. Brifely, one colony of *E.coli* HB2151 containing pCANTAB-WBP was cultured in LBG (LBG 2% glucose) at 37 °C for 16-18 h. The 1 mL of mixture was transferred into 10 mL of LBG (LB 2% + glucose + 80 mg/mL of ampicillin) and incubated at 30 °C for 1.15 h. The culture was centrifuged at 1500 rpm for 20 min at room temperature. The pellet was resuspended in 10 mL of LB containing 1 mM IPTG and 80 mg/mL of ampicillin and incubated at 30 °C for 26 h. The mixture was centrifuged at 1500 rpm for 20 min. The protein was purified from the supernatant using Sephadex™ G-25 (GE Healthcare Bio-Sciences AB, Sweden) connected to an AKTAprime plus (GE Healthcare Bio-Sciences AB, Sweden). The obtained protein from eluting fractions were checked by 15% SDS-PAGE and kept at -80 °C.

3. Immobilization of WBP on the electrode

The procedure was followed by Loyprasert-Thananimit *et al*., 2012. Brifely, a gold electrode was polished using alumina slurries and cleaned through sonication in deionized water, follow by electrochemical etching in 0.5M H_2SO_4 using a potential from 0 to 1.5 V versus an Ag/AgCl reference electrode with a scan rate of 0.1 V/s for 25 scans. Then, the cleaned gold electrode was immersed in 250 mM thioctic acid solution at room temperature for 12 h (Limbut *et al*., 2006). During this steps a self-assembled monolayer onto the electrode surface. The gold electrode was then immersed in an EDC: NHS solution (EDC 1% (v/v), NHS 2.5% (v/v) in 0.05 M phosphate buffer pH 5.00 with 0.05 M KCl) for 5 h. Then 20 µL of 200 µg/ml of WBP in phosphate buffer pH 7.4 was immediately placed on the electrode surface and left overnight at 4 °C. Finally the electrode was immersed in 10 mM 1-dodecanethiol ethanolic solution for 20 min to block any pinholes on the electrode surface, before connected to the biosensor system.

4. Detection of WSSV

A flow injection technique was set-up as shown in Figure 1. The modified gold electrode (working electrode), a custom made Ag/AgCl (reference electrode) and platinum wire auxiliary electrode (auxiliary electrode) were placed in flow cell and connected to the Autolab PGSTAT30 electrochemical impedance analyzer and potentiostat/ galvanostat (Eco Chemmie B.V., Netherlands). Impedance was measured at +200 mV dc potential with the ac amplitude of ±10 mV. The Eco Chemie software, Frequency Response Analyzer (FRA 4.9.005), was used to monitor the impedance. To investigate the change of impedance, the imaginary part of the impedance (Z'') was monitored (Bart et al., 2005). When the WSSV was injected and bound to the immobilized WBP molecules on the working electrode causing the impedance to increase. The impedance change (ΔZ'') was measured to determine the signal. The experiment was done in three replicate per each concentration. The conditions used in the flow injection biosensor were followed by Loyprasert-Thananimit *et al*., 2012.

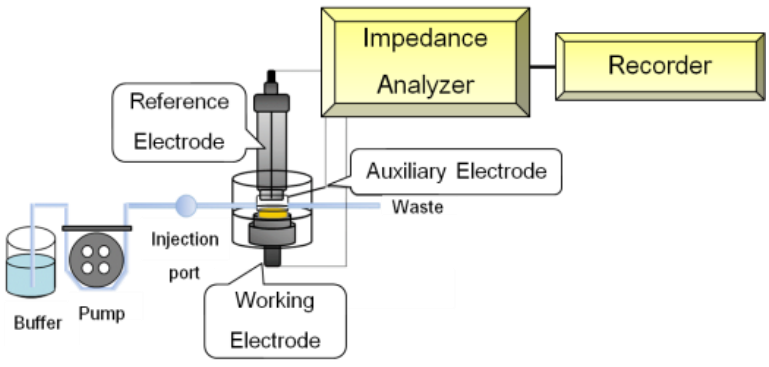

Figure 1 Schematic diagram showing the label-free impedimetric biosensor

RESULTS AND DISCUSSION

1. Production of WBP from pCANTAB-WBP *E. coli* (HB2151)

WBP was produced form pCANTAB-WBP in *E. coli* (HB2151) and purified. WBP was 3 kDa on 15% SDS-PAGE as shown in Figure 2.

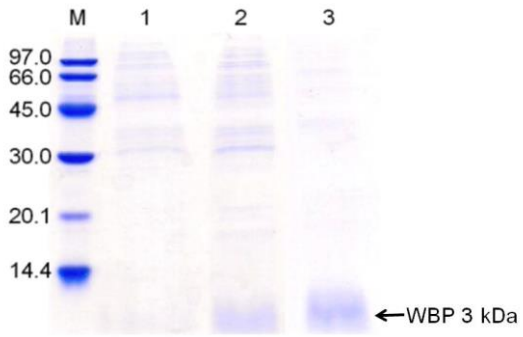

Figure 2 15% SDS-PAGE (M) low molecular weight standard marker, (1) lysate of bacteria containing pCANTAB-WBP plasmid before induction with 1 mM IPTG, (2) lysate of bacteria containing pCANTAB-WBP plasmid after induction with 1 mM IPTG, (3) WBP purified protein (3 kDa).

2. Sensitivity

The impedimetric signal due to WSSV binding was found to be linearly dependent on the WSSV amount in the range of $1.6x10^{1}$-$1.6x10^{5}$ copies, with an r value of 0.997. The impedance response (ΔZ'') increase with the virus concentration as shown in Figure 3.

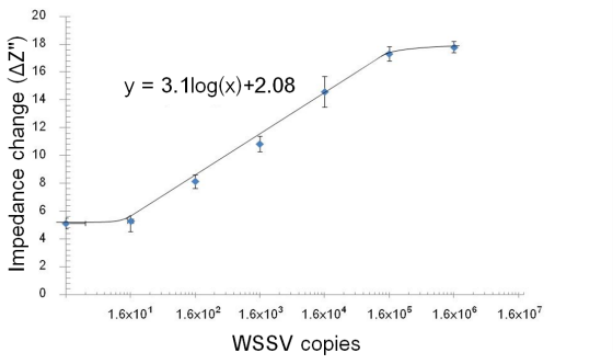

Figure 3 Impedance change (ΔZ'') response according to the binding between WBP and WSSV versus the standard copies of WSSV.

CONCLUSION

In conclusion, WBP was produced and purified for used to immobilize on the gold electrode surface and tested for binding to WSSV by developed label-free impedance biosensor. The binding between WBP and WSSV was monitored by impedimetric change (ΔZ'') and was found to increase with the virus concentration. The biosensor could detect WSSV in the range of $1.6x10^{1}$-$1.6x10^{5}$ copies. Another advantage of this biosensor is simple to operate, reliable, reproducible and may be applied in areas of shrimp cultivation.

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