UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF A SURFACE PLASMON RESONANCE CHIP BASED VIRAL ASSAY FOR PSEUDORABIES VIRUS INFECTION

PARISA HONARI

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By

PARISA HONARI

Thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

November 2011

To

MY DEARLY BELOVED FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman: Assoc. Prof. Zeenathul Nazariah Allaudin, PhD

Faculty: Veterinary Medicine

Studies were conducted to establish a viral based biochip using Surface Plasmon Resonance (SPR) technology for detection of Pseudorabies virus (PrV. To establish such biochip, series of optimizations were carried out. Different Ligands including P-PrV, PrV/PAb, PrV/gC-mAb and Vero cells were immobilized on the surface of the selected appropriate carboxylated chips. The optimum conditions of immobilization and the level of immobilization achievement were different based on the molecular biochemistry of each ligand. Higher immobilization level belonged to PrV/PAb (13560 RU) and the lowest immobilization level to Vero cells (514 RU). A Regeneration scheme was obtained for P-PrV, PrV/gC-mAb, PrV/PAb and Vero cells each associating with specific analyte. Additionally, a non-enveloped virus, Infectious Bursal Disease Virus (IBDV), an enveloped virus , Classical Swine Fever Virus (CSFV), and a non-viral biomolecule, insulin, were adopted in this regeneration scheme for comparison purposes. The

results of regeneration showed when an enveloped virus as one of the interacting partners was considered, acidic solution or a combination of acidic and chelating solution were the most effective regeneration solutions. Whilst, a combination of basic and chelating solution was found to be the most effective regeneration solution when a non–enveloped virus (IBDV) was one of the interactant partners. Thus, the optimum regeneration conditions were dependent on the nature of analyte-ligand bond. Prior to application of established chip in antiviral and mutant assays, the chip was validated in terms of specificity. The chip immobilized with PrV/gC-mAb was found to be specifically sensitive to P-PrV with the highest interaction of ~582 RU. Also, the sensitivity of this chip with limit of detection (LOD) of 1.5 PFU enabled the early detection of virion in supernatants collected from cell culture even prior to formation of cytopathic effect (CPE).

Following the validation of biochip, several viral assays for detection of virus in crude samples, screening of virus-antibody interactions, antiviral investigation and detection of mutant virus (BUDR7-PrV) were designed using bovine lactoferrin (BLf) as the investigated antiviral. The potential inhibitory effect of BLf as an antiviral against PrV was revealed through both conventional cell based method and SPR chip based method. BLf with the concentrations of 15 mg/mL and 10 mg/mL was able to significantly reduce the plaque size up to 34.37% and 27.84%, respectively. Besides, the number of plaques was significantly reduced to 13.35% and 9.47% in the presence of BLf with the concentrations of 15 mg/mL and 10 mg/mL, respectively. Accordingly, the results obtained from SPR analysis confirmed BLf inhibitory

effect. Based on the SPR analysis, RU was reduced 18.32% when using 15 mg/mL of BLf, while 10.13% reduction of RU was obtained when using 10 mg/mL of BLf. Mutant detection was successfully conducted via established SPR chip. Conventional Polymerase Chain Reaction (PCR) and Western Blot (WB) analyses were employed to investigate the existence of gC gene and its protein. The comparison of SPR based assays and WB revealed that SPR assay is fast, reliable, label free, automated and yet required lower (lesser 10 folds) amount of samples. In conclusion, this study has successfully established an optimized SPR protocol for chip based viral assays specifically with the advantage of early virus detection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PEMBANGUNAN PENGUJIAN VIRUS BERASASKAN PERMUKAAN CHIP PLASMON RESONANS untuk VIRUS PSEUDORABIES JANGKITAN

Oleh

PARISA HONARI

November 2011

Pengerusi: Profesor. Madya Zeenathul Nazariah Allaudin, PhD

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Kajian telah dijalankan untuk membangunkan biochip berasaskan virus yang menggunakan teknologi Permukaan Plasmon Resonans (SPR) untuk pengesanan virus Pseudorabies (PrV) dan jangkitannya. Untuk membangunkan biochip tersebut, beberapa siri pengoptimuman telah dijalankan. Ligands berbeza termasuk P-PrV, PrV/PAb, PrV/gC-mAb dan sel Vero telah dikekalkan di permukaan cip carboxylated terpilih yang sesuai.. Keadaan optimum immobilization (pengekalan) dan tahap pencapaian immobilization (pengekalan) adalah berbeza berdasarkan biokimia molekul setiap ligand. Immobilization (Pengekalan) lebih tinggi milik PrV/PAb (13.560 RU) dan tahap immobilization (pengekalan) terendah ialah sel-sel Vero (514 RU). Skim (penjanaan semula) telah diperolehi bagi P-PrV, PrV/gC-mAb, PrV/PAb dan sel Vero untuk setiap “analyte” dengan sekutu khususnya.

Selain itu, virus bukan menyelubungi, Infectious Bursal Disease Virus (IBDV), virus yang menyelubungi, Clasical swine Fever Virus (CSFV), dan biomolekul bukan virus (insulin) telah diterima pakai dalam skim penjanaan semula ini untuk tujuan perbandingan. Keputusan untuk penjanaan semula menunjukkan apabila virus yang menyelubungi digunakan sebagai salah satu daripada biomolekul yang berinteraksi, campuran berasid atau kombinasi campuran berasid dan "chelating" adalah campuran penjanaan semula yang paling berkesan. Sementara itu, gabungan alkali dan campuran "chelating" telah didapati sebagai campuran penjanaan semula yang paling berkesan apabila virus bukan menyelubungi (IBDV) menjadi salah satu biomolekul yang berinteraksi. Oleh itu, keadaan optimum penjanaan semula bergantung kepada jenis ikatan analyte-ligand. Sebelum pengujian cip ke atas antivirus dan mutan, cip telah disahkan dari segi ketepatan. Cip yang telah ditetapkan dengan PrV/gC-mAb didapati sensitif khusus kepada P-PrV dengan interaksi tertinggi pada ~ 582 RU. Selain itu, kepekaan cip ini dengan LOD 1.5 PFU membolehkan pengesanan awal virion dalam supernatan yang diambil daripada kultur sel walaupun sebelum pembentukan kesan cytopathic (CPE).

Berikutan pengesahan biochip, beberapa ujian virus untuk mengesan virus dalam sampel mentah, saringan interaksi antibodi virus, antivirus penyiasatan dan pengesanan virus mutan (BUDR7-PrV) telah direka dengan menggunakan lembu Laktoferin (BLf) sebagai antiviral yang disiasat. Potensi kesan pencegahan daripada BLf sebagai antiviral terhadap PrV dibuktikan melalui kaedah konvensional berasaskan sel dan cip berasaskan SPR. BLf

dengan kepekatan 15 mg/mL dan 10 mg/mL mampu untuk mengurangkan saiz plak kepada 34.37% dan 27.84%.Selain itu, bilangan plak berkurangan ketara kepada 13.35% dan 9.47% dalam kehadiran BLf dengan kepekatan 15 mg/mL dan 10 mg/mL.. Oleh itu, keputusan yang diperolehi daripada analisis SPR mengesahkan kesan pencegahan BLf. Berdasarkan kepada analisis SPR, RU dikurangkan 18.32% apabila menggunakan 15 mg/mL BLf, manakala pengurangan sebanyak 10.13% RU diperolehi apabila menggunakan 10 mg/mL BLf. Pengesanan Mutant telah berjaya dijalankan menerusi cip SPR yang dibangunkan. Reaksi polimeras berantai (PCR) dan analisis Western Blot (WB) telah digunakan untuk menyiasat kewujudan gC gen dan protein. Perbandingan ujian antara SPR dan WB mendedahkan bahawa SPR cepat, boleh dipercayai, label bebas, automatik dan jumlah sampel yang diperlukan lebih rendah (kurang 10 kali ganda). Kesimpulannya, kajian ini telah berjaya mewujudkan protokol optimum SPR untuk ujian khusus virus berasaskan cip dengan kelebihan pengesanan awal virus.

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APPROVAL SHEETS

I certify that an Examination Committee has met on **16th November 2011** to conduct the final examination of **Parisa Honari** on her thesis entitled "**ESTABLISHMENT OF A SURFACE PLASMON RESONANCE CHIP BASED VIRAL ASSAY FOR PSEUDORABIES VIRUS** " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

PARISA HONARI

Date: 16 November 2011

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LIST OF ABBREVIATIONS

A	Analyte
Ab	Antibody
AB	Lewis acid-base
ACV	Acyclovir
ADV	Adenovirus
ANOVA	Analysis of variance
APS	Ammonium persulfate
ATV	Antibiotic-Trypsin-Versine
BLf	Bovine Lactoferin
CC50	Cytotoxicity Concentration of 50%
Cox-A	Coxsackie virus
CPE	Cytopathic Effect
CSFV	Classical Swine Fever Virus
Da	Dalton
DAB	chromogen (3-3-diamino benzidine hydrochloride)
DAF	Decay-Accelerator Factor
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDC	1-Ethyl-3-(3-Dimethylaminopropyl) Carbodimide
EDTA	Ethylene diamine tetra acetic acid
EL	Electrostatic force
ELISA	Enzyme Linked Immunoassay
EN	Enterovirus
EV	Echovirus
exp	Exponential function
FC	Fellow Cell
FCS	Fetal Calf Serum
FCV	Feline Calicivirus
FHV	Feline Herpes Virus
g/L	gram per liter
HAV	Hepatitis A Virus
HBS	Hepes Buffer Saline
HBV	Hepatitis B Virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HPV	Human Papilomavirus
HRP	Horseradish peroxides

HRV	Human Rhinovirus
HSV	Herpes Simplex Virus
H1N1	Human Influenza Virus
HV	Hanta Virus
IBDV	Infectious Bursal Disease Virus
IFC	Integrated Flow Chanel
IUDR	Iododeoxyuridine
kDa	kiloDalton
Lf	Lactoferin
Lg	Ligand
LLOQ	Lower limit of quatification
LOD	Limit of detection
LOQ	Limit of quantification
LW	Vanderwaals forces
M	Molar
mAb	Monoclonal antibody
mg	Milligram
min	Minute
mM	Millimolar
mg	Milligram
mg/mL	Milligram per milliliter
NCBI	National Center for Biotechnology Information
ng	NanoGram
NHS	*N*-hydroxysuccinimide
PAb	Polyclonal Antibody
PCR	Polymerase Chain Reaction
pI	Isoelectric point
P-PrV	Parental Pseudorabies Virus
PR	Puerto Rico Virus
PrV	Pseudorabies Virus
PV	Poliovirus
PVDF	Polyvinylidene fluoride
R_e	Regeneration Efficiency
RIA	Radioimmunoassay
RNA	Ribonucleic Acid
RO	Regeneration Optimization
RPMI	Roswell Park Memorial Institute medium
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RU	Response Unit
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

Sec	Second
SPR	Surface Plasmon Resonance
TEM	Transmission Electron Microscopy
TFT	Trifluorothymidine
TMV	Tabaco Mosaic Virus
µg	Microgram
mL	Microliter
U/mL	Unit per milliliter
ULOQ	Upper limit of quantification
V	Volt
v/v	Volume per Volume
WB	Western Blot
w/v	Weight per Volume

CHAPTER 1

INTRODUCTION

Surface Plasmon Resonance (SPR) is a sensitive optical detection method for characterizing macromolecular interactions using the evanescent wave phenomenon. It measures the changes in refractive index close to a sensor surface (McDonnell, 2001). This technique was introduced in the early 1990s as the essential technology in affinity biosensors (Englebienne *et al.*, 2003). SPR spectroscopy provides a powerful approach to investigate macromolecule interactions in real time without using labels (Cooper, 2003; Liedberg *et al.*, 1995).

Application of SPR based biosensor is increasingly popular in wide variety of fields such as proteomics, drug screening, medical diagnostics and food safety due to its unique characteristics. It offers the opportunity to make comparisons of specificity, kinetics and affinities of macromolecule interactions, with the possibility of high` throughput analysis and low sample consumption (Yuk & Ha, 2005). Biacore AB (Uppsala, Sweden), the most commonly used SPR biosensor, is the first commercial instrument which was released in 1990 (Rich & Myszka, 2000). A Biacore instrument consists of an SPR detector, an exchangeable sensor chip, and an integrated liquid handling system for the exact transport of sample to the adsorption and detection spot (Torreri *et al.*, 2005). The sensor chip usually consists of a carboxylated dextran matrix covalently attached to a gold surface on which one of the reactant molecules (ligand) is immobilized (Torreri *, et al.*, 2005).

The integrated microfluid system allows analyte (the other reactant partner) in the tested solution to pass over the sensor surface in a continues, pulse free and controlled flow that maintains constant analyte concentrations at the sensor surface (Torreri *, et al.*, 2005). Currently, Biacore technology has been applied in different virology fields as a sensitive reliable diagnostic tool through immobilization of DNA, RNA, protein (usually antibody-antigen), virus particles and in few studies whole virus (José M. Casasnovas & Springer, 1995 ; Guo *et al.*, 2005; Richalet-Sécordel *et al.*, 1996; K. M. Wilson *et al.*, 2006).

Unlike the currently available viral assays such as enzyme-linked immunosorbent assay (ELISA), cell culture, polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), real-time PCR and radioimmunoassay (RIA), SPR makes it possible for us to monitor interactions in real time and further quantifying and qualifying analysis with a low limit of detection.

Although SPR technology is widely used in recent years, there is no specific report on SPR application for detection of Pseudorabies virus (PrV) infection. Also, there is still no record on direct immobilization of enveloped virus on SPR chips. Since the use of gene deleted virus vaccine is becoming a common practice in field, a system for detection of mutant viruses is crucial. However, commercial units for mutant screening in vaccine development are still lacking. Moreover, there is an urge in development of new antiviral agents through a fast, reliable and sensetiv technique such as SPR.

In this study, we took advantage of Biacore SPR technology to design several viral assays for detection of enveloped virus in crude samples, screening of virus-antibody interactions, antiviral investigation and detection of mutant viruses. The virus of interest was PrV which is a member of *Alphaherpesvirinae* subfamily and the etiological agent of Aujeszky's disease. A glycoprotein C (gC) PrV mutant (BUDR7-PrV) was chosen for mutant detection. A reliable fast assay was designed to differentiate the gC positive and gC negative strains without relying on DNA sequencing.

The investigated antiviral agent was bovine lactoferrin (BLf), known as an iron-binding protein which closely resembled in structure to the plasma iron-transport protein transferrin. Although it is proposed that lactoferrin has an antiviral activity, its biological role has not been recorded yet. In this study, by using SPR technology, the biological role and mechanism of action of BLF was predicted.

Therefore the objectives of this study can be summarized as below:

1) To establish an optimized SPR based diagnostic chip for detection of PrV and its infection.

2) To establish a regeneration scheme for reusability of the optimized biosensor chip.

3) To validate the SPR chip in terms of specificity for PrV detection, analyzing analyte-ligand interaction and designing of an antiviral assay.

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