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An aptamer based surface plasmon resonance biosensor for the detection of bovine catalase in milk

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ABSTRACT

In this research, we report the development of an aptamer based SPR biosensor for the detection of catalase in milk samples with minimal sample preparation. A biotin tagged aptamer was immobilized onto a gold surface by affinity capture. A limit of detection (LOD) in the nanomolar range (20.5 nM, RSD: 15.2%) was found and a dynamic range of 15–1000 nM was established for catalase in buffer and the aptamer showed good specificity toward catalase. This biosensor has the potential to be used in the detection of catalase in milk samples, a key indicator of mastitis disease in milk.

1. Introduction

Surface Plasmon resonance (SPR) has emerged as a powerful technique in bioanalytical and chemical analyses. It allows for the detection of biomolecules with high sensitivity and selectivity and gives valuable information about the kinetics of bio-interactions.

Aptamers are an emerging class of ligands for SPR based biosensors known as aptasensors. Aptamers are ssDNA that binds to a large variety of targets such as proteins and small molecules. They are an alternative to antibodies and are predisposed for SPR applications due to their stability and small size relative to antibodies. Aptamers are also cheap to produce and can be tagged with a variety of biomolecules and functional groups that allow for easy immobilization onto the SPR surface. Moreover aptamers do not suffer from a loss of activity upon tagging which can occur with antibodies. To date there have been a number of aptasensors developed to detect and quantify proteins. In 2005 Tombelli developed an RNA aptamer based biosensor for quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) to detect HIV-1 Tat protein (Tombelli et al., 2005). Lee et al. developed an aptamer based SPR sensor for the detection of retinal binding protein (RBP4) using an aptamer based system (Lee et al., 2008). They reported a limit of detection (LOD) of 75 nM which was comparable to the conventional immunoassay based methods. A sandwich style SPR aptasensor assay was developed in 2009 for the detection of IgE protein allowing for a detection limit of 2.07 ng/ml (Kim et al., 2009). More recently, a SPR aptasensor was developed for the rapid detection of H5N1 avian influenza with a detection range of 0.128–1.28 hemagglutinin unit (HAU) (Bai et al., 2012). In 2012, Chang et al. developed a SPR based biosensor for the detection of interferon-gamma (IFN-γ) achieving a linear dynamic range of 0.3–333 nM and a detection limit down to picomolar range (Chang et al., 2012). Aptamer based SPR biosensors offer a convenient platform for disease screening and one area that could be of great interest is in the screening for bovine mastitis in milk samples. SPR based biosensors would provide a quick, cheap and sensitive method for screening this disease with minimal sample preparation.

Mastitis is a disease which causes inflammation of the udder tissue in cattle and is the most common disease affecting the dairy industry today. This disease can increase the somatic cell count in milk (an indicator of milk quality) and is caused by a number of pathogens including *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus spp*. A number of methods have been developed to determine the total somatic cell count which can include any bacterial cells (Viguier et al., 2009). In 2009 Koskinen et al. used a PCR based assay to identify mastitis pathogens in milk samples (Koskinen et al., 2009). A chip based method was recently developed to detect mastitis pathogens using a ligation detection reaction (Cremonesi et al., 2009). An online conductivity system for the detection of mastitis has also been developed which

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identified a number of sub clinical quarters (Lansbergen et al., 1994). Recently protein bio indicators of mastitis disease in milk samples have been identified in proteomic studies (Boehmer, 2011). One possible protein identified as a bio indicator of mastitis disease is catalase (Lansbergen et al., 1994). Catalase enters the milk by somatic cells and catalase levels correlated with bulk milk somatic cell counts (Hamed et al., 2008). Futo et al. recently developed an amperometric based biosensor for the detection of catalase in milk samples by measuring the degradation of hydrogen peroxide indirectly (Fűtő et al., 2012). As catalase is a large analyte, SPR would be a convenient method for detecting this analyte in milk.

In this study we developed a highly sensitive and specific SPR based aptasensor for the detection of catalase protein in milk samples. An aptamer selected from our previous study was used as the transduction element of the biosensor (Ashley et al., 2012). This biosensor was used to measure the catalase directly in milk samples and could allow for rapid determination of mastitis disease in milk.

2. Experimental

2.1. Materials and apparatus

Catalase, casein, β lactoglobulin and bovine albumin proteins were purchased from Sigma Aldrich (Singapore) and dissolved in 10 mM HEPES, 100 mM KCl, 1 mM EDTA and 0.5% BSA buffer pH 7.4 (HKE-BSA). Standardized milk samples were purchased from a local supplier (Meiji, Singapore). For the formation of the self-assembly monolayer (SAM), 11-mercaptoundecanoic acid (11-MUA) and 2-mercaptoethanol (2-ME) were purchased from (Sigma Aldrich, Singapore) and dissolved in absolute ethanol. N-hydroxysuccinimide and ethyl 1-Ethyl-3-(dimethylaminopropyl) carbodiimide were also purchased from Sigma Aldrich (Singapore). Streptavidin was purchased from Merck Chemicals (Singapore) and dissolved in 20 mM sodium acetate buffer pH 5.0. Biotinylated aptamer ligand of sequence CTTCTG CCC GCC TCC TTC CGACCTAG CAGTGGACA TGTGGCAGGGTG AAGTGGCA TCGTCGGAGAC GAG ATAGGC GGA CAC T-3′ biotin was purchased from (1st Base, Singapore) and reconstituted in 10 mM HEPES, 100 mM KCl, 1 mM EDTA, pH 7.4 (HKE) buffer. Aptamers were renatured using a Bio-Rad DNA engine thermocycler (Bio-Rad, Singapore) by heating to 94 °C for 10 min and then cooling down at rate of 0.5 °C/s to room temperature. SIA AU kits containing bare gold chips were purchased from Biacore (Sweden). All experiments were carried out on the Biacore T3000 (Biacore, Sweden). For all experiments, the machine was purged with run buffer prior to analysis. In the immobilization of streptavidin, the flow buffer was 20 mM sodium acetate buffer pH 5.0 and the flow rate was set to 5 μl/min. The flow buffer was then changed to HKE buffer pH 7.4 at a flow rate of 5 μl/min for the immobilization of the aptamer ligand. For the optimization of the catalase assay and real milk analysis, a flow buffer of 10 mM HEPES, 100 mM KCl, 1 mM EDTA and 0.5% BSA buffer pH 7.4 (HKE:BSA) buffer was used and the flow rate was set to 10 μl/min. All buffers were degassed by **Sonication** prior to analysis.

2.2. Preparation of the chip surface

The gold slides were treated with piranha solution, rinsed with water and ethanol and dried with nitrogen. The gold slide was submerged in a solution containing 11-MUA 1 mM and 2-ME 4 mM in absolute ethanol for 24 h. The slide was then rinsed with water and ethanol and dried by nitrogen. The gold slide was then inserted into the chip and docked into the T3000. For the immobilization of streptavidin, 20 mM sodium acetate buffer pH 5.0 was used as a flow buffer at a flow rate of 5 μl/min. A solution of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was mixed (1:1) and 50 μl was injected onto channels 1 and 2 to activate the carboxylic acid groups on the 11-MUA and 2-ME. 50 μl of streptavidin 50 μg/ml was then injected onto the sensor. The online response was used to characterize the sensor surface.

The remaining unreacted ester groups were blocked using 50 μl of 1 M ethanol amine (pH 8.5). For the biotin tagged DNA, the flow buffer was changed to HKE buffer and the flow rate was set to 5 μl/min. 50 μl of biotin tagged DNA (10 μM) was injected and captured onto the surface of channel 2 only.

2.3. Catalase assay and real sample analysis

For the catalase assay and real sample analysis, HKE:BSA buffer was used as the flow buffer and for each cycle the flow rate was set to 10 μl/min. 50 μl of catalase (10–1000 nM) was injected after 180 s. After the end-point of each injection, a dissociation time of 360 s was applied, followed by injection of 50 μl of 0.1 M NaOH and 45 mM glycine in 1.2% ethanol regeneration buffer (see Supplementary data, Fig. S1). A further waiting time of 180 s was applied after each injection to allow the baseline to stabilize. A calibration plot with linear regression of the relative response at 480 s against catalase concentration was plotted and the LOD was determined as three times the standard deviation ($S/N=3$) of three blank samples.

Milk samples were prepared by spiking with different concentrations of catalase (1–0.01 μM). Spiked samples were centrifuged and the supernatant was transferred to a new vial and filtered through a 0.45 μm filter. For each cycle the flow rate was again set to 10 μl/min and injection of 50 μl of each sample (62.5–1000 nM) was performed after 180 s. After each injection of catalase, a dissociation time of 360 s was applied followed by injection of 50 μl of regeneration buffer. A further wait time of 180 s was applied after each injection to allow the baseline to stabilize. The relative response plots were obtained by subtracting the milk only negative control. A graph with linear regression of the relative response at 480 s against catalase concentration was plotted.

3. Results and discussion

3.1. Preparation of the chip surface

A bare gold Biacore chip was coated with a mixture of 11-MUA (1 mM) and 2-ME (4 mM) in absolute ethanol for 24 h. A mixture of thiols was used to achieve easy amine coupling of the protein and to reduce steric hindrance of the protein toward the SAM. The immobilization of streptavidin and the affinity capture of the biotin tagged aptamer were monitored by measuring the relative response (RU). The online response plots for the immobilization of streptavidin and the affinity capture of the biotin tagged aptamer are shown in Fig. 1. For the immobilization of streptavidin, the flow buffer used was sodium acetate (pH 5.0). This is due to the near neutral *pI* value of streptavidin and an attempt to encourage an electrostatic interaction between the activated ester groups on the SAM and the protein leading to preconcentration of the protein onto the surface of the chip.

The tagged DNA was captured by injecting 50 μl at a concentration of 10 μM with a flow rate of 5 μl/min. In general there was no improvement in the response at concentrations >10 μM of tagged aptamer. The binding buffer was changed to HKE buffer (HEPES/KCl/EDTA, pH 7.4) as the sodium acetate buffer would facilitate degradation of the aptamer through hydrolysis in acidic