Biosensor for bacteria detection from powdered milk

Contamination of food by pathogenic bacteria is a serious threat to human health and thus biosensors for fast and accurate food quality control are extensively studied.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) based biosensor was developed to detect Salmonella Typhimurium in dairy products. Direct label-free detection of bacteria by using a capture antibody was further improved utilizing bio-catalyzed precipitation. For control samples the limit of detection (LOD) was 102 CFU/mL and for real samples (powdered milk) LOD was 103 CFU/mL, demonstrating a high sensitivity of the biosensor.

Introduction

Currently used methods for *Salmonella* detection include culturing, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Farka *et al*. 2016). These methods are generally time-consuming, they require complex sample pre-treatment and trained personnel, thus more robust and easy-to-use methods are being actively developed.

Surface Plasmon Resonance (SPR) is a well-established method utilized to measure binding affinity and kinetics. Innovative Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can perform measurements in an exceptionally wide angular range (40-78 degrees) and at more than one wavelength, thus allowing a wide range of applications from small molecule interactions to real-time detection of bacteria, cells and viruses.

MP-SPR is an unparalleled method for biosensor development, whether the sensor is based on MP-SPR detection or whether you are developing new portable (point-of-care) biosensors. Amongst many advantages of MP-SPR, high sensitivity and label-free detection are the most prominent.In addition, it allows the development of a sensor directly on the material-of-choice: metal electrodes for electrochemistry, plastics for well-plate assays, cellulose for printed biosensors, glass for traditional chemistry, nanoparticles for SERS, etc. avoiding assay transfer step. Sensor slides can be easily modified *in situ* and *ex situ* providing a wide range of possibilities for functionalization (CVD, ALD, spin coating, dip coating, etc.). Furthermore, MP-SPR allows measurements of complex/crude samples (milk, 100% serum, urine, sea-water, etc.), unlike many traditional SPR instruments. After MP-SPR measurement, the sensor surface can further be characterized with other methods such as AFM. This is enabled thanks to the oil-free operation of MP-SPR, using a prism coated with an optical elastomer.

Materials and methods

In this study, the measurements were performed with BioNavis MP-SPR Navi™ 210 VASA instrument at 20 µL/min flow-rate. Carboxymethyl dextran (CMD 3D) sensor slides were cleaned with a solution of 2 M NaCl and 10 mM NaOH for 5 min, followed by the activation of carboxylic groups using a mixture of EDC (200 mM) and NHS (50 mM) for 7 min. The capture antibody (and BSA for the reference channel) was introduced (10 µg/mL in 50mM acetate buffer, pH 4.5) before blocking the remaining reactive groups by ethanolamine (1 M, pH 8.5, 5 min). For additional blocking, BSA (2 mg/mL in HBS-P) and 1% powdered milk in HBS-P were injected.

The powdered milk (Laktino) was diluted in the HBS-P buffer to the concentration of 1%. Varying concentrations of enterica (subsp. enterica serovar Typhimurium) cells were added to the sample after culturing and heat treatment (80°C/30min). Concentrations of treated bacteria are expressed as CFU/mL corresponding to viable cells before the treatment.

The bacteria were injected for 10 min, followed by 10 min injection of the horseradish peroxidase antibody conjugate (HRP-Ab2) and 10 min of precipitation substrate solution (HRP) (Figure 1). The bio-catalyzed reaction converted 4-chloro-1-naphthol to insoluble benzo-4-chlorocyclohexadienone. The limit of detection (LOD) was evaluated based on the signal-tonoise ratio, where the measurable minimum signal level has to be three times higher than the noise level.

Figure 1. *Salmonella* detection from powdered milk using Multi-Parametric Surface Plasmon Resonance (MP-SPR). Binding of horseradish peroxidase antibody 2 (HRP-Ab2) specifically on *Salmonella* and formation of precipitate.

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Results and discussion

The biosensor was first developed on a Biacore 3000 SPR instrument where LOD of 104 CFU/mL was achieved. To further improve the biosensor, a bio-catalyzed precipitation reaction was selected for sensitivity enhancement. However, horseradish peroxidase requires the use of ethanol. The Biacore instrument is not compatible with alcohols, unlike BioNavis MP-SPR instruments which provide excellent resistance to organic solvents. Thus, MP-SPR Navi™ 210A VASA was selected for further research.

Using the MP-SPR instrument, a biosensor was successfully developed and the bio-catalyzed precipitation enhanced the limit of *Salmonella* detection (LOD) from 104 to 102 CFU/mL (Figure 2). The binding level was high in the channel containing the capture antibody and the bacteria, whereas only a minor amount of precipitate was formed in the reference channel (BSA treated). *Salmonella* binds multiple HRP-Ab2 conjugates which improves sensitivity of the biosensor exponentially with the increasing concentration of microbes. The HRP-Ab2 conjugate is specific to *Salmonella*, also providing improved selectivity compared to direct binding assay. The crossreactivity of the developed biosensor was tested with Gram-negative bacteria *E. coli* K-12 which showed negligible binding. Optical microscopy and AFM images of an MP-SPR sensor slide were used as reference, and both confirmed bacteria attachment and precipitate formation on the biosensor surface (Figure 3).

The total analysis time by MP-SPR was 60 minutes which is significantly shorter than other methods used for detection of bacteria, such as cultivation (~days), ELISA (~10 h) and PCR (~hours).

The mechanisms underlying the binding of bacteria to surfaces is important in order to develop biosensors and coatings in material research. MP-SPR has been utilized to characterize functional "self-defence" anti-microbial implant coatings (Cado *et al.*, 2013). Coating releases anti-microbial peptides by stimulation with pathogens. Here, MP-SPR is used to measure the build-up of a multilayer and quantifies adsorbed mass.

See also cancer cells (MCF7) detection from blood using a target peptide in MP-SPR instrument, Application Note #154.

Conclusions

MP-SPR proved itself as a highly sensitive and selective biosensor to detect bacteria from food samples. In addition, it is invaluable for assay development in food and environmental safety, clinical diagnostics, border and process control.

The key benefits to use MP-SPR in biosensor development are:

- Compatibility with organic solvents
- Easy modification of sensor surfaces
- Capability to work with crude samples

Original publication:

Farka *et al.*, Analytical Chemistry, Vol. 88 (23), 2016

Reference:

Cado *et al.*, Advanced Functional Materials Vol. 23 (38), 2013

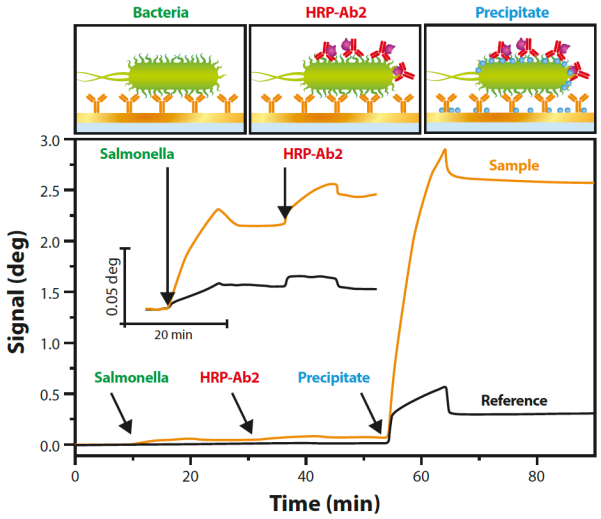

Figure 2. Detection of *Salmonella* is based on antibody (HRP-Ab2) binding on bacteria and bio-catalyzed precipitation by horseradish peroxidase. Binding of several HRP-Ab2 on one bacteria cause exponential amplification of the signal.

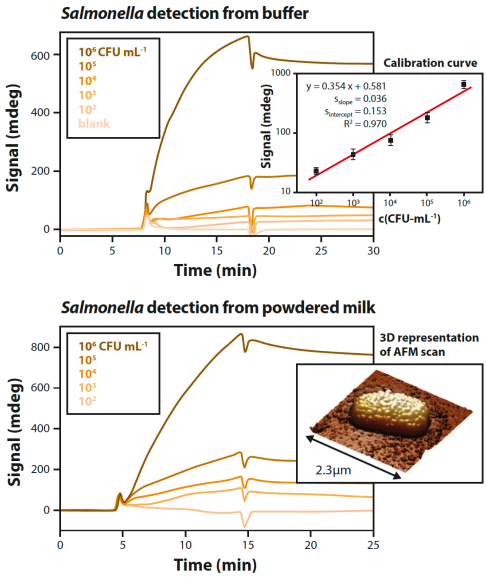

Figure 3. Bio-catalyzed precipitation caused by different concentrations of bacteria in buffer (on top) and bacteria detection from real-sample, powdered milk (below). AFM images confirmed bacteria attachment and formation of precipitates.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI and 210A VASA with additional wavelength -L

Sensor surface: CMD, Au, other metal or inorganic coating

Software: MP-SPR Navi™ Controller, DataViewer and TraceDrawer™ for MP-SPR Navi™

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