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Chitosan-nickel film based interferometric optical fiber sensor for label-free detection of histidine tagged proteins

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ABSTRACT

An interferometric fiber sensor for detection of hexa-histidine tagged microcin (His-MccS) is reported and experimentally demonstrated. This intermodal fiber sensor is implemented by a no-core fiber (NCF) functionalized with chitosan (CS)-nickel (Ni) film for direct detection of small peptide: microcin. The fiber intermodal sensor relies on the refractive index modulations due to selective adsorption event at the chitosan (CS)-nickel (Ni) film. Owing to the strong affinity between Ni^{2+} ions and histidine, the immobilized Ni^{2+} ions in the chitosan film were utilized as binding agents for the direct detection of hexa-histidine tagged microcin. A comparative study in relation to different target size was conducted: full proteins trypsin, bovine serum albumin (BSA) and human serum albumin (HSA), with high histidine content on their surface and His-MccS (peptide, 11.6 kDa), have been employed for sensor evaluation. Results have shown selectivity for His-MccS relative to trypsin, BSA and HSA. The most telling contribution of this study is the fast detection of small biomolecule His-MccS compared to standard detection procedures like SDS-PAGE and western blot. The proposed sensor exhibits His-MccS detection sensitivity of 0.0308 nm/(ng/ml) in the range of (0–78) ng/ml with concentration detection limit of 0.8368 ng/ml.

1. Introduction

In the field of molecular biology recombinant expression of proteins by cloning is state of the art and has been in practice for the last few years (Baneyx, 1999; Sørensen and Mortensen, 2005). Detection and purification of these proteins are done efficiently by genetically incorporating an epitope tag. This process involves addition of amino acid sequences to the terminal ends of the protein, which act as specific sites for binding partners like antibodies (Brizzard and Chubet, 2001). One of the most used methods for protein tagging involves genetically adding poly-histidine tags to the terminal ends of the protein. The strong affinity between the histidine tag and a divalent metal ion such as nickel (Ni^{2+}) enables the purification of proteins with a high degree of specificity using metal affinity chromatography (Arnold, 1991; Lichty et al., 2005; Schmitt et al., 1993). Subsequently, detection and quantification of His-tagged proteins after purification will be carried out so that the yield can be determined. SDS_PAGE is a standard technique used for identification of the His-tagged protein after purification. Western blot technique is also used in some cases when there is availability of anti-His-tag antibodies (Oshannessy et al., 1995; Zentgraf et al., 1995). However, the characterization of His-tagged proteins using these standard procedures is time consuming and tedious. A fast detection method for specific detection of His-tagged proteins could therefore simplify the procedure dramatically. Hence, alternative methods have been developed for detection of His-tagged proteins such as surface plasmon resonance (SPR) sensors and nano-material (eg: silicon nanowires) based field effect transistors (Noor and Krull, 2014; Sigal et al., 1996). Even though the responses of these sensors are faster than the standard procedures, they suffer from issues like complex fabrications and high costs. On top of that, functionalizing of Ni^{2+}-NTA (nitrilotriacetic acid) film onto solid surfaces requires complex chemical strategies (Richard et al., 2003; Tinazli et al., 2005). This necessitates the design of a new sensor which is robust, relatively easier to fabricate and cost-effective without compromising on the performance.

In this work, we propose a fiber optic interferometric sensor

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functionalized with CS-Ni film for the detection of hexa-histidine tagged protein. The CS-Ni film not only provides specific binding sites for direct immobilization of histidine tagged protein but also offers 'soft' surface to prevent protein from denaturation and preserves their bio specific activity. Fiber optic sensors have been used extensively in the field of sensing due to their unique characteristics such as low cost, immunity to electromagnetic interference, high sensitivity, ease of multiplexing and remote sensing capability (Lee et al., 2012). Among fiber optic sensors, those based on intermodal interference have gained considerable interest on account of their compact structure, large dynamic range and high stability (Lee et al., 2012) and have been reported extensively (Gu et al., 2015, 2013; Silva et al., 2012; Tou et al., 2014). Therefore, owing to all these intrinsic properties, the proposed intermodal sensor is able to provide fast detection and enable both quantitative and kinetic recordings of the specific analyte-probe binding events.

In our effort, the sensor is designed for the detection of histidine tagged microcin (His-MccS). Microcins are low molecular weight antibacterial peptides produced by enterobacteria (Duquesne et al., 2007) and the synthesis is activated under conditions of stress such as nutrient depletion (Chiuchiolo et al., 2001; Fomenko et al., 2001). Microcins belong to the class of bacteriocins and exhibit antibacterial mechanisms, especially against infections with gram-negative bacteria (Cotter et al., 2013), and are believed to assist intestinal microbiota against possible takeover by competing enterobacteria (Duquesne et al., 2007). Thus, they offer promise in the development of novel and efficient antibiotics (Gaggero et al., 1993; Metlitskaya et al., 1995). Amongst the gut flora, probiotic *E. coli* G3/10 produces a novel microcin that is named microcin S (MccS) (Zschuttig et al., 2012). The gene clusters for microcin S synthesis involves four genes grouped in a single operon. The clustered genes for microcin S is composed of (i) microcin gene (*mcsS*) (ii) self-immunity gene (*mcsI*) that encodes the protein that nullifies the microcin activity thereby protecting the producing strain, (iii) microcin export and external secretion genes (*mcsA* and *mcsB*).

In order to selectively detect His-MccS, the NCF is functionalized with a suitable bioreceptor. The immobilization of the bioreceptor has to be done with utmost care, since the sensor's performance is largely governed by the stability of the bioreceptor immobilization. Chitosan based electrochemical biosensors have been reported previously and extensively involve electrochemical methods for detection and quantification of the target species (Barsan et al., 2014; Luo et al., 2004; Yang et al., 2012). Singh et al. have demonstrated an electrochemical based chitosan-nickel oxide film biosensor for the detection of cholesterol (Singh et al., 2011). Even though these chitosan composite based electrochemical biosensors show good sensitivity and selectivity to target molecules like glucose, cholesterol, catechol etc., their ability to be used for protein detection is hindered by the need to use mediators since many proteins analytes are not intrinsically capable of being redox partners and this complicates the sensor fabrication process (Chaubey and Malhotra, 2002). In this work we have utilized a CS-Ni film based optical intermodal sensor for detection of hexa-histidine tagged proteins. Intermodal sensors in addition to the advantages mentioned above, monitor the binding of protein to the CS-Ni functionalized sensor surface by tracking the ambient refractive index changes and hence can detect any histidine tagged protein which binds on to the CS-Ni film. The CS-Ni film acts as a bioreceptor since hexa-histidine tag in the protein selectively binds to divalent metal ions like Ni^{2+} (Ahmed et al., 2006; Wu et al., 2006). Chitosan is selected as the chelating agent for Ni^{2+} ion owing to its film forming ability, biocompatibility and ability to bind divalent metal ions like Ni^{2+} (Raghunandhan et al., 2016). In our previous work, we have reported a fiber optic interferometric sensor functionalized with self-assembled polyelectrolyte multilayers of chitosan/poly acrylic acid for detection of Ni^{2+} ions (Raghunandhan et al., 2016). Amine groups present in same or different chitosan polymer chains can bind to Ni^{2+} through inter or intramolecular bonds (Guibal, 2004; Rhazi et al., 2002). Upon capturing His-MccS by the CS-Ni film, the sensor will effectively transduce refractive index change of the CS-Ni film into a quantifiable interferometric fringes signal. The sensor's response to a full protein: trypsin, which inherently contains histidine residues on the surface, was verified to evaluate the specificity of the sensor towards His-MccS. Our work features the first experimental evidence about reliable and fast detection of His tagged proteins with interferometric fiber optic biosensors.

2. Methods

2.1. Sensor fabrication

The fiber optic interferometric sensor was fabricated by splicing 20 mm of no-core fiber (NCF) (Prime Optical Fiber Corporation) between single mode fibers (SMFs) (8.2/125 μm SMF-28) using a Sumitomo Type 39 electric arc splicer.

2.2. Sensor functionalization

2 g of chitosan was dissolved in 4% acetic acid solution to prepare a 2% chitosan solution and the pH was adjusted to 6. To 20 ml of this chitosan solution 0.1 g of $NiCl_2$ was added and the solution was stirred continuously at room temperature for 24 h and subsequently filtered to remove the excess $NiCl_2$. Based on our previous work (Raghunandhan et al., 2016), 10 mM $NiCl_2$ would completely occupy the binding sites of chitosan and a higher number of nickel ions translate to a wider sensing range. Hence to ensure the complete coverage of the binding sites of chitosan, a $NiCl_2$ concentration slightly higher than 10 mM (approx. 30 mM) was chosen. Prior to the functionalization process, NCF sensor was first immersed for 60 min in piranha solution (30% Hydrogen peroxide and concentrated sulphuric acid (98%) in 1:3 ratio) and subsequently immersed in distilled water and finally dried under a stream of nitrogen gas. Piranha treatment cleaned the NCF surface of organic impurities and hydroxylated the fiber surface. Next, the treated fiber was placed in a custom-made polydimethylsiloxane (PDMS) micro-channel and chitosan-nickel blend was injected into the channel and dried overnight in a vacuum oven at 60 °C.

2.3. Microcin S expression and purification

The recombinant expression of microcin S was performed in 250 ml of LB broth where exponentially grown *E. coli* Top10 cells with the recombinant plasmid pBbE8k-pBAD-McsS-His were grown to an optical density of 0.7 – 1, and their expression was induced fully by 0.2% L-arabinose ($C_5H_{10}O_5$, Sigma-Aldrich, item no. A3256) at 37 °C and 225 rpm for 4–5 h. Subsequent centrifugation at 4000×g for 20 min yielded cell pellets and homogenization was carried out using Emulsiflex-C3 homogenizer (Avestin, Inc.). Ni-NTA (Ni^{2+}-nitrilotriacetate) column (Qiagen) was used for the purification of microcin S (His-MccS) under native conditions. Washing was carried out with 50 mM Na_3PO_4, 300 mM NaCl, and 10 mM imidazole (pH 7.4). Elution was performed with 50 mM Na_3PO_4, 300 mM NaCl, and 500 mM imidazole (pH 7.4). Since the estimated molecular weight of microcin S is approximately 12 kDa, the eluate was first added to Amicon Ultra-15 Centrifugal Filter Unit, Millipore and concentrated using a 30-kDa molecular mass cut-off membrane. The concentrated fractions containing the microcin S was further concentrated using a 3-kDa cut-off membrane. After this, the resulting retentate was collected and the microcin S concentration was determined using Quant-iT™ protein (ThermoFisher Scientific, Cat. no.: Q33210) for subsequent use.