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Biosensors based on modularly designed synthetic peptides for recognition, detection and live/dead differentiation of pathogenic bacteria

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ABSTRACT

Rapid and sensitive detection of bacterial pathogens is critical for assessing public health, food and environmental safety. We report the use of modularly designed and site-specifically oriented synthetic antimicrobial peptides (sAMPs) as novel recognition agents enabling detection and quantification of bacterial pathogens. The oriented assembly of the synthetic peptides on electrode surfaces through an engineered cysteine residue coupled with impedimetric detection facilitated rapid and sensitive detection of bacterial pathogens with a detection limit of 10^2 CFU/mL for four bacterial strains including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*). The approach enabled differentiation between live and dead bacteria. The fabrication of the sAMPs functionalized surface and the importance of the sAMPs orientation for providing optimum recognition and detection ability against pathogens are discussed. The proposed methodology provides a universal platform for the detection of bacterial pathogens based on engineered peptides, as alternative to the most commonly used immunological and gene based assays. The method can also be used to fabricate antimicrobial coatings and surfaces for inactivation and screening of viable bacteria.

1. Introduction

Bacterial infection is one of the most threatening public health problems of significant social and economic impact. According to recent Centers for Disease Control and Prevention (CDC) reports, from 2009 to 2013, the number of illness cases caused by foodborne bacterial infection is 29,337. *Salmonella spp.*, *Campylobacter spp.* and *Escherichia coli* (*E. coli*) are the primary pathogens responsible for the major foodborne bacterial pathogen outbreaks in US (Centers for Disease Control and Prevention, 2013, 2014a, 2014b, 2015). The large number of reported outbreaks requires rapid and sensitive detection methods that can provide fast screening of samples for microbial contamination.

The most commonly used methods to detect bacteria are the colony counting assays with analysis of colony forming units (CFU), immunological assays and polymerase chain reaction (PCR). The CFU assay is labor intensive and time-consuming with some standardized procedures requiring up to 7 days (de Boer and Beumer, 1999). Immunological assays require expensive materials and involve labeling and multiple washing steps (Beumer and Brinkman, 1989; Palumbo et al., 2003). The nucleic acid probe-based technique is expensive and requires specialized facilities (Cai et al., 2014). Moreover, nonviable cells and single naked DNA, extraction and degradation of nucleic acids, and/or the direct inhibition of the PCR may lead to false positive/negative responses (Wilson, 1997). Except for the CFU assay, most methods cannot differentiate live/dead bacteria.

In this work, we report a rapid, sensitive and label free detection of pathogenic bacteria, using synthetic antimicrobial peptides (sAMPs) as novel biorecognition elements for the detection and differentiation of live/dead bacteria. The method provides a simplified and cost effective alternative to the most commonly used immunological and DNA based procedures. As compared to other biorecognition entities, modularly designed sAMPs provide several advantages including: facile, low cost and large scale production, high stability even in harsh environments (Friedrich et al., 1999; Rydlo et al., 2006), ease of functionalization enabling design of

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specific binding sites and sequences with improved recognition function. We took advantage of these unique characteristics to develop a biosensor using sAMPs with site specifically engineered amino acids for the detection of bacterial pathogens. To our knowledge, this is the first platform for bacteria detection based on site specifically oriented attachment of synthetic sAMPs with selective microbial recognition ability.

To design the sensor, we use previously reported cationic Multidomain Peptides (MDPs) which can be modularly designed to form various protein secondary structures and demonstrate tunable, structure-dependent antimicrobial activities (Dong et al., 2007; Xu et al., 2015; Yang et al., 2014). MDPs have a general formula of $K_x(QL)_yK_z$ where the middle domain contains variable number (y) of alternating hydrophilic (glutamine, Q)-hydrophobic (leucine, L) repeating units terminated with two oligolysine (K) domains. The relative ratio of the repeating number (i.e. *x*, *y*, *z*) of each domain dictates the molecular secondary structures of MDPs and further their antimicrobial activity (Xu et al., 2015; Yang et al., 2014). In this work, $WK_3(QL)_6K_2$ was primarily used for bacterial detection to validate the designed sensor platform. In the sequences, tryptophan (W) was appended at the N-terminus to allow an accurate concentration measurement. The peptide forming mixed secondary structures of beta sheets and random coils (Fig. 1A) has been tested for its antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* in broth medium with minimum inhibitory concentration (MIC) value in the range of 5–20 μM (Xu et al., 2015; Yang et al., 2014). In this work, we will demonstrate the use of these peptides as recognition sites for bacterial detection. To design the sensor, the peptides were modified with an external cysteine (C) residue that allows targeted attachment and site-specific orientation to a gold surface using the affinity of cysteine for gold (Fig. 1). We use electrochemical impedance spectroscopy (EIS) as transduction technique to quantify the interaction between the synthetic sAMPs and bacteria. The effect of the orientation of peptides on the electrode surface was investigated by comparing the impedimetric responses of the sensors modified with the engineered sAMPs with and without cysteine. Sensors modified with peptides without significant antimicrobial activity were also tested to link the peptide sequence with the antimicrobial activity and establish the recognition ability against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. The method was employed to quantify four bacterial strains (both gram-positive and gram negative) and to differentiate between live and dead bacteria.

This work demonstrates the potential of engineered sAMPs for rapid and sensitive detection of bacteria. This study also lays the foundation for a new technology of using designed peptides for fabricating functionalized surfaces with antimicrobial activity for biodetection and potential therapeutic applications (e.g. antimicrobial coatings).

2. Materials and methods

2.1. Reagents and equipment

Potassium ferricyanide, ACS grade was purchased from Fisher Scientific (Hampton, NH, USA), 20 mM Tris–HCl buffer, pH=7.5 was prepared from Trizma™ hydrochloride purchased from Sigma (St Louis, MO, USA). Glutaraldehyde, 50% in water, reagent grade was purchased from Spectrum Chemical (Brunswick, NJ, USA). Deionized (DI) water from Direct-Q system (Millipore, Billerica, MA) with a resistivity of 18.2 MΩ cm was used to prepare all solutions. All electrochemical experiments were conducted using a CHI 920C analyzer (CH Instruments, Austin, TX, USA). FMOC-amino acids and resin were purchased from NovaBiochem. Peptide synthesis grade solvents, deprotection reagent and piperidine were purchased from Sigma. Trifluoroacetic acids and diethyl ether were from Fisher.

2.2. Preparation of the engineered peptides

The preparation of the engineered peptides followed the same procedure as previously described (Xu et al., 2015). For this study, we have used the $WK_3(QL)_6K_2$ peptide unit at which we have added a cysteine residue. Briefly, $WK_3(QL)_6K_2G_3C$ was synthesized on a PS3 peptide synthesizer (Protein Technologies Inc., Tucson, AZ) using standard FMOC (fluorenylmethyloxycarbonyl)-solid phase peptide synthesis method. The sequence without significant antimicrobial activity, $WK_2(QL)_6K_2G_3C$, was used as control peptide. $WK_3(QL)_6K_2$ was used as a negative control to determine the role of the cysteine residue in the immobilization process. The sequences $WK_2(QL)_6K_2$ and $CE_2(QL)_6E_2$ (glutamic acid, E) were used to demonstrate the role of antimicrobial activity in bacteria-sAMP recognition process. The sequence and the design characteristics of all peptides used in this study are listed in Table S1.

2.3. Fabrication and characterization of the AMP based biosensor

The biosensor was developed on a gold disk electrode (1.6 mm diameter, MF-2014, Bioanalytical Systems, Inc., West Lafayette, IN). Prior to use, the gold disk electrode was polished using 0.3 μm

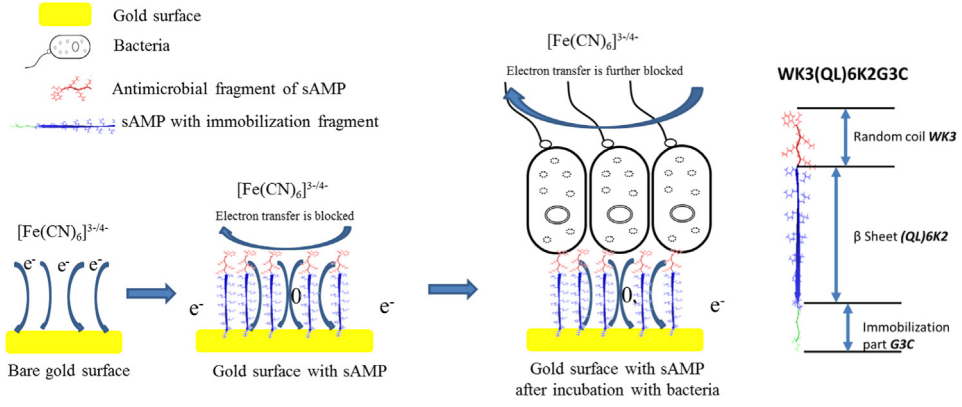

Fig. 1. A. Design and sensing principle of the cysteine modified sAMP impedimetric biosensor. B. Sequence and orientation of the active $WK_3(QL)_6K_2G_3C$ peptide on the gold surface.