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Olfactory receptor-based polypeptide sensor for acetic acid VOC detection

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ABSTRACT

Rapid detection of food-borne pathogens in packaged food products can prevent the spread of infectious diseases. This study investigates the application of novel sensing material that is sensitive to specific indicator volatile organic compound (VOC) related to *Salmonella* contamination in packaged meat. Specifically, the objective was to develop an olfactory receptor-based synthetic polypeptide sensor for the detecting acetic acid in low concentrations and at room temperature. Synthetic polypeptide was deposited on a quartz crystal microbalance (QCM) electrode and was evaluated for detecting acetic acid at 10–100 ppm. Developed sensor exhibited repeatable response to a particular concentration of acetic acid and displayed reproducibility among multiple sensors during acetic acid detection. Mean estimated lower detection limits of these sensors were about 1–3 ppm and linear calibration models showed linear relationships. Thus, the QCM sensors exhibited a great potential for detecting low concentrations of acetic acid at room temperature and can be used in the sensor array for packaged meat spoilage and contamination detection.

1. Introduction

Food safety is critical for a healthy society. Olfactory sensing offers a promising method for rapid and intelligent detection of food-borne pathogens in packaged beef. Gaseous metabolites of pathogen trapped in packaged food can be detected using various sensors. Studies have found the potential of olfactory sensing for the detection of spoilage or/and contamination in food products [1–7]. These studies have evaluated the application of olfactory sensor system for the detection of volatile organic compounds (VOCs) present in the headspace of food products as a valid indicator to the microbial population such as spoilage bacteria, *Salmonella typhimurium*, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Aspergillus carbonarius*. These studies have utilized metal-oxide or conducting polymer-based sensor materials for VOC detection.

Mammalian olfactory system can distinguish various odorant molecules with high accuracy and at very low concentrations (ppb–ppt). Olfactory sensors that mimic mammalian olfactory receptors would have a good sensitivity and selectivity to low molecular weight VOCs. Few prior studies have utilized the biological olfactory system based components such as olfactory receptors, odorant binding proteins (OBPs), and synthetic olfactory peptides for sensor development [8–14]. Among various components, the olfactory receptors can be an effective tool as a sensing material [15]. Recently, researchers [10,16–18] have utilized the olfactory receptors (protein) derived from the animals as a sensing material for detecting low molecular weight VOCs. In addition to the olfactory receptors, carbon-based sensing materials have also been used for detecting VOCs [19,20].

Kim et al. [16] developed a single-walled carbon nanotube-field effect transistor based bioelectronic sensors, functionalized with human olfactory receptor (hOR2AG1) to detect amyl butyrate, butyl butyrate, propyl butyrate, and pentyl valerate. The developed sensors obtained sensitivity to pM–μM concentration of odorous gases. Although this approach is promising, the olfactory receptors need to be extracted through elaborate processes before their use for sensor applications. Alternatively, the amino acid residues/polypeptide chains derived from olfactory receptors can be used as sensing materials for VOC sensing.

Mascini et al. [21] developed a biomimetic olfactory receptor-based sensor for detecting dioxins at very low concentrations (ppb) in food products. Research is ongoing in polypeptide-based gas sensor development due to its inherent advantages in comparison to the application of olfactory system-based proteins (which might be laborious and time consuming to get predictable protein expressions). Wu et al. [12] developed polypeptide sequences and evaluated their relative sensitivities to different gases including acetic acid, ammonia, ethanol, acetone, etc. The group's previous [14] and recent [8] studies indicated that the polypeptide-based sensor might have been developed to detect higher concentrations of VOCs (>1000 ppm). Our previous research [22,23] indicated that acetic acid is one of the VOCs that could be related to *Salmonella* contamination in packaged beef. Therefore, there was a need for further systematic evaluation of polypeptide-based sensor for detecting acetic acid at low concentrations.

Based on this motivation, our goal was to evaluate few polypeptide sequences for its ability to detect organic compounds of a particular

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functional group. This study presents the systematic development and evaluation of polypeptide-based biosensor as sensing material for the detection of acetic acid at low concentration. The overall objective of this study was to develop and evaluate QCM based-biomimetic olfactory sensors for sensing low concentrations (low ppm) of specific indicator VOC (acetic acid) associated with *Salmonella* contamination in beef. Once these sensors are developed, they will be part of a sensor array (of a custom developed sensor system) that will be used for packaged meat contamination detection.

2. Materials and methods

2.1. Sensing material and polypeptide synthesis

The olfactory receptor binding site residues (amino acids) were used as sensing material. Wu et al. [12] used simulation programs for determining polypeptide sequence that would be selective to acetic acid. Although the polypeptide sequence, RVNEWVIC was found to be selective, Wu [12] did not evaluate the sensitivity of the material. This study evaluates the polypeptide sequence 'RVNEWVIC' for its sensitivity to acetic acid at low concentrations. The sensors were also tested to determine its sensitivity to other gases (an alcohol and a ketone that are also found in the headspace of packaged meat), to reconfirm the specificity of the polypeptide sequence to acetic acid.

In this study, the polypeptide sequence was synthesized by Fmoc method using a microwave polypeptide synthesizer with standard Fmoc-protected amino acids. The Fmoc protected amino acids were purchased from Peptide International, KY and ChemPep, Inc., FL. 1-Hydroxybenzotriazole (HOBT), and a coupling reagent were purchased from AK Scientific, CA. All the solvents of spectroscopic grade were purchased from Fisher Scientific and were used without further purification. Polypeptide was synthesized using a microwave assisted polypeptide synthesizer (Liberty, CEM Corporation, Matthews, NC) in 0.1 mmol scale. 'Fmoc-Cys(Trt)-Clear-Acid Resin' was used as the solid support, and a mixture of HOBT/O-Benzotriazole-N,N,N′,N′-tetramethyl-uronium-hexafluoro-phosphate (HBTU), polypeptide coupling reagents was used for coupling in five-fold excess. Except amino acid 'Arg', all the amino acids were coupled once using 20 W microwave for 5 min at a temperature of 50 °C. 'Arg' was subjected to double coupling at 25 °C without the use of microwave. The Fmoc deprotection was accomplished by treating with 5% (by weight) of piperazine in dimethylformamide (DMF) and diisopropylethylamine dissolved in N-methylpyrrolidone was used as the activator base. Cleavage from the solid support was performed using microwave for 35 min. A mixture of trifluoroacetic acid (TFA), triisopropylsilane, and water (95%–2.5%–2.5%) was used as the cleavage cocktail. The crude polypeptide was precipitated using ice-cold ether and purified by semi-preparatory reverse phase-high performance liquid chromatograph (RP-HPLC).

The conditions for semi-preparatory HPLC operation were — column: Vydac C_{18} HPLC column (238TP152022), and eluent: linear gradient of 0–70% acetonitrile in water over 60 min with both solvents containing 0.1% TFA at flow rate of 7 mL/min. The conditions for analytical HPLC operation were: column: Vydac C_{18} HPLC analytical column (238TP5415), and eluent: linear gradient of 0–70% acetonitrile in water over 40 min with both solvents containing 0.1% TFA at flow rate of 1.5 mL/min. Purity of the polypeptide sequence was >90%. Olfactory receptor binding site residues were used as sensing material to detect acetic acid. The polypeptide sequence was part of transmembrane domain of the olfactory receptor (OR1E1, Swiss-Prot P30953) and hydrophobic in nature [24].

2.2. Deposition process

Polypeptide sequence was deposited on the QCM crystal by the process of self-assembled monolayer (SAM). The thiol group of cysteine (amino acid) at the end of both polypeptides enables monolayer formation [25,26] on the gold electrode of the QCM crystal. The AT-cut QCM crystals (International Crystal Manufacturing, OK) were 10 MHz resonant frequency crystal with a polished gold electrode. Quartz diameter was 13.7 mm, while the gold electrode diameter was 5.1 mm. QCM crystals were cleaned prior to the deposition process. The QCM crystals were pre-rinsed with acetone, methanol, and deionized water, and dried. QCM crystals were then cleaned using piranha solution (20 μL, 30% H_2O_2: conc. H_2SO_4, 1:3 v/v) to remove the organic materials on the surface. Finally, the QCM crystals were rinsed with deionized water, followed by 200 proof ethanol, and dried with nitrogen.

Polypeptide solution (5 mM) was prepared using acetonitrile as the solvent. QCM sensors were developed using two different procedures (methods) in order to determine the effect of dispersed volume on the QCM sensor's sensitivities and film characteristics. In each method, polypeptide was deposited on both sides of the QCM crystal. In method-I, 10 μL was deposited; while in method-II, 2 μL was deposited on each side of the crystal; which resulted in total of 20 μL deposition in method-I and 4 μL deposition in method-II. Three sensors were developed using each of method-I and -II. Sensors were then incubated for 48 h at room temperature. After the deposition process, sensors were rinsed with acetonitrile to remove excess polypeptides and dried with nitrogen. QCM sensors were stored in vacuum dessicator until further use. The schematic of the sensor development process and analysis is shown in Fig. 1.

2.3. Experimental set-up and gas sensing characterization

Fig. 2(a) presents the schematics of the experimental set-up used for gas sensing characterization of the QCM sensors. Sensor was placed in a 140 mL hexagonal sensing chamber and was connected to the oscillator circuit (Standard Oscillator, International Crustal Manufacturing, OK) and that was in-turn connected to the frequency counter (model: 1823A, BK Precision Inc., CA) through a BNC cable.

The VOC to be sensed was generated in a 5 L three-necked flask using liquid injection method [27]. The desired acetic acid gas concentration was acquired in ppm. QCM sensor sensitivities to VOCs were measured in terms of change in frequency. The gas sensitivities were tested at room temperature and with atmospheric air as a reference gas. Gas sensing time cycle used was as follows: initial purging time = 120 s; stabilization time in air = 300 s; gas introduction time = 30 s; gas retention time = 300 s; and final purging time = 180 s. Raw sensor response data (frequency) was processed using a user interactive Visual C++ 'QCM Sensor Program'. Based on the time and frequency from the raw data, the reference frequency (F_{ref}) was estimated (Eq. (1)) and the data was normalized (Eq. (2)):

$$F_{ref} = Average\left[F_i\right]_{Intb-60s}^{Intb-130s} \tag{1}$$

$$F_{norm} = \left[F_{ref} - F_i\right]_{SPte}^{Intb-130s} \tag{2}$$

where, *Intb* = start time of the gas introduction time (s), F_{norm} = normalized frequency (Hz), F_i = frequency at time *i* (Hz), and *Spte* = second/final purging time end (s). From the normalized data, the blank frequency (F_{bl}), standard deviation of blank ($F_{bl\text{-}sd}$), average sensitivities (*Ave. Sens.*), and maximum sensitivities (*Max. Sens.*) were calculated using the following equations (where, *GRte* = gas retention time end in seconds):

$$F_{bl} = Average\left[F_{norm}\right]_{Intb}^{Intb-50s} \tag{3}$$

$$F_{bl-sd} = Standard\ Deviation\left[F_{norm}\right]_{Intb}^{Intb-50s} \tag{4}$$