Biosensors and Bioelectronics 26 (2011) 3103–3109

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Odorant binding protein based biomimetic sensors for detection of alcohols associated with *Salmonella* contamination in packaged beef

Sindhuja Sankaran[a,b], Suranjan Panigrahi[a,c,*], Sanku Mallik[d]

[a] *Agricultural and Biosystems Engineering, North Dakota State University, Fargo, ND 58108, USA*
[b] *Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA*
[c] *Electrical and Computer Engineering Technology, Purdue University, 401 N Grant Street, West Lafayette, IN 47907, USA*
[d] *Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58108, USA*

ARTICLE INFO

Article history:
Received 7 May 2010
Received in revised form 25 July 2010
Accepted 29 July 2010
Available online 27 August 2010

Keywords:
Food safety
Piezoelectric sensors
Odorant binding protein
LUSH
Alcohols

ABSTRACT

Detection of food-borne bacteria present in the food products is critical to prevent the spread of infectious diseases. Intelligent quality sensors are being developed for detecting bacterial pathogens such as *Salmonella* in beef. One of our research thrusts was to develop novel sensing materials sensitive to specific indicator alcohols at low concentrations. Present work focuses on developing olfactory sensors mimicking insect odorant binding protein to detect alcohols in low concentrations at room temperature. A quartz crystal microbalance (QCM) based sensor in conjunction with synthetic peptide was developed to detect volatile organic compounds indicative to *Salmonella* contamination in packaged beef. The peptide sequence used as sensing materials was derived from the amino acids sequence of *Drosophila* odorant binding protein, LUSH. The sensors were used to detect alcohols: 3-methyl-1-butanol and 1-hexanol. The sensors were sensitive to alcohols with estimated lower detection limits of <5 ppm. Thus, the LUSH-derived QCM sensors exhibited potential to detect alcohols at low ppm concentrations.

1. Introduction

Food safety is important for the welfare of the society. Artificial olfactory sensing provides a promising technique for rapid and intelligent detection of food-borne bacteria in packaged meat products. Gaseous metabolites of bacteria trapped in the headspace of the packaged food product can be detected using olfactory sensors. Studies have found the potential of olfactory sensing for the detection of spoilage or/and contamination in food products (Concina et al., 2009; Bianchi et al., 2009; Balasubramanian et al., 2005, 2008; Panigrahi et al., 2006a,b; Mayr et al., 2003). Our research group aims in developing multiple sensing techniques for the detection of *Salmonella typhimurium* in packaged beef. One of our major thrusts is to develop and evaluate novel sensing materials to detect some of the specific indicator volatile organic compounds (VOCs) at room temperature. The study in this paper focuses on developing sensors for some of the alcoholic compounds, that will be a part of sensor system (having an array of sensors), which in future, will be used for contamination studies.

Biological olfactory system has the ability to identify various odorant molecules accurately and at very low concentrations (ppb-ppt). The olfactory biosensors mimicking the biological olfactory system can be highly sensitive and selective to odorant compounds. Some researchers have employed biological olfactory system based biomaterials such as olfactory receptors, odorant binding proteins (OBPs), and synthetic olfactory receptors based polypeptides (Lu et al., 2009; Vidic et al., 2006; Liu et al., 2006; Sung et al., 2006; D'Auria et al., 2004; Wu et al., 2001; Lin et al., 2001; Wu and Lo, 2000) for gas sensing.

OBPs are components of the olfactory signaling (Kim and Smith, 2001; Breer, 1997; Buck, 1996) that could be used as potential candidates for sensor development. OBPs are low molecular weight soluble proteins, serving as carriers for conveying odorants through the nasal mucus in the olfactory system. The reversibility of odorant–OBP complex with dissociation constants in the micromolar range (Hou et al., 2005) enables the utilization of OBPs as preferable sensing materials in an olfactory sensor system. Very few studies have explored the possibility of utilizing OBPs as sensing materials (Hou et al., 2005; D'Auria et al., 2004). D'Auria et al. (2004) extracted OBP from dog's nasal epithelium to develop OBP based biosensor for detecting pyrazine. The biosensor was sensitive to 10–100% of 0.2 mM pyrazine solution in the presence of 0.75 mg mL^{-1} of dog OBP. Similarly, Hou et al. (2005) found that the recombinant rat OBP (1F) based sensor exhibited a decrease in resistance (from 1.18 MΩ to 25 kΩ) on exposure to isoamyl acetate (odorant). The odorant response of the recombinant rat OBP (1F) based sensor was measured based on the change in sensor electrical property (change in resistance) using non-faradaic electrochemical spectroscopy.

* Corresponding author. Tel.: +1 765 494 6908; fax: +1 765 496 1354.
E-mail address: spanigr@purdue.edu (S. Panigrahi).

0956-5663/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.bios.2010.07.122

The molecular interactions that define the alcohol-binding site in the structure of LUSH (an odorant binding protein that binds to alcohols in *Drosophila*) have been identified (Kruse et al., 2003). LUSH is known to exhibit a high sensitivity and selectivity to alcohols. No study has been performed for evaluating LUSH as suitable sensing material for the detection of alcohols in sensor applications. Thus, OBP such as LUSH was used as a potential sensor material for detecting alcohols.

Presently, simulated polypeptide amino acid residues based on mammalian olfactory receptors are being developed as a possible sensing material for VOC sensing. The application of synthetic peptide sequence as a sensing material can be an advanced technique for developing a highly sensitive and selective biosensor to detect low concentrations of VOCs. The application of biological olfactory system based peptide sequences selective to a particular odorant would provide a good stability and reproducibility during the fabrication of the sensors (Mascini et al., 2005; Wu et al., 2001). In addition, they would be relatively selective (similar to protein) and relatively less expensive (Mascini et al., 2005). Lu et al. (2009) developed an array of sensors (based on four polypeptide sequences and two conducting polymers) and evaluated their sensitivities and selectivities to various gases (such as acetic acid, butyric acid, ammonia, and benzene). The study indicated the potential of such sensing materials for detecting and distinguishing VOCs.

The present study investigates the application of odorant binding protein based sensing material for its ability to detect alcohols. Our previous work (Bhattacharjee et al., 2010) indicated that 3-methyl-1-butanol and 1-hexanol are among the few gaseous compounds that could be released during *Salmonella* contamination in packaged beef. The sensor performance was evaluated for these two alcohols. To the best of our knowledge, no work has reported the application of biosensor based on LUSH to detect lower concentrations of alcohols in the context of sensor development and food contamination.

The overall goal of the present research was to develop and evaluate odorant binding protein (LUSH) based piezoelectric sensors for detecting low concentrations of alcohols (3-methyl-1-butanol and 1-hexanol) at room temperature.

2. Materials and methods

2.1. Sensing material

The alcohol-binding site of odorant binding protein, LUSH has been identified (Kruse et al., 2003). The sensing material used in this study to detect alcohols was derived from odorant binding protein, LUSH. Their studies (Kruse et al., 2003) demonstrated Threonine (Thr 57) and Phenylalanine (Phe 64) as two of the several amino acid residues that bind with alcohols such as ethanol and butanol. Though there have been studies performed on determining the LUSH OBP sensitivity to alcohols (Kruse et al., 2003; Kim and Smith, 2001), to the best of our knowledge, there has been no application of LUSH binding site as a sensing material in an olfactory sensor.

Based on their studies (Kim and Smith, 2001; Kruse et al., 2003), a hypothesis was postulated that if the amino acid residues (Threonine and Phenylalanine) were a part of peptide sequence, the sensors would exhibit good sensitivity to alcohols. The peptide sequence used in this study was selected such that the binding amino acid residues (Threonine, T and Phenylalanine, F), were a part of the sequence (Supplementary information, Fig. S1). The peptide sequence used in this study was SLMAGTVNKKGEFC, which is the part of entire amino acid sequence (primary structure) of LUSH protein. Cysteine (C) was added to the end of the peptide sequence to promote self-assembled monolayer formation. The peptide sequence was custom ordered from Creative Peptides, NY. The purity of the peptide sequence as reported by Creative Peptides based on high performance liquid chromatography (HPLC) analysis was about 93%. The white lyophilized peptide powder was stored at −20 °C freezer until further used.

2.2. Deposition process

The peptide solution (5 mM) containing a sequence of OBP LUSH was prepared using dimethyl sulfoxide (DMSO) as the solvent. The peptide sequence was deposited in the quartz crystal microbalance (QCM) crystal through self-assembly. The thiol group (cysteine) at the end of the peptide enables self-assembly (Love et al., 2005; Ulman, 1991). The QCM crystals (International Crystal Manufacturing, OK) were procured with a resonant frequency of 10 MHz and with polished gold electrodes. The diameters of quartz and gold electrodes were 13.7 mm and 5.1 mm, respectively. A special QCM crystal holder was fabricated such the QCM crystal could be held firmly in a stable position, during the cleaning and deposition process. The crystals were cleaned with acetone, methanol, and deionized water, and dried with nitrogen. Piranha solution (20 μL, 30% H_2O_2:conc. H_2SO_4, 1:3 v/v) was used to clean the gold electrode. The crystal was finally rinsed with deionized water, followed by rinsing with 200 proof ethanol (dehydrated or anhydrous ethanol), and dried with nitrogen.

Four sensors were developed to verify the reproducibility of the sensor response among the sensors. 5 μL peptide solution was deposited on the either side of gold electrode of the quartz crystal. After deposition on one side, the sensors were stored for 48 h at room temperature. Following storage period with the peptide solution, the sensors were rinsed with deionized water to remove excess peptides from the surface and were dried with nitrogen. This procedure was repeated on other side of the QCM crystal. The frequency change corresponding to the amount of peptide deposited was 50 Hz. The sensors were stored in vacuum desiccator.

2.3. Experimental set-up

Gas sensing characterization of the sensors was performed using specially designed experimental set-up, gas sensing chamber, and data acquisition system (Fig. 1a). The QCM crystal was placed in a 140 mL hexagonal sensing chamber and connected to the oscillator circuit (Standard Oscillator, International Crustal Manufacturing, OK). Agilent 53131A frequency counter with 0.001 Hz resolution at 1 s gate time was used to characterize the gas sensitivity. The frequency counter was connected to the computer via a GPIB-USB converter.

The gas concentration was generated in a 5 L three-necked flask using liquid injection method. The desired gas concentration (C, ppm) using liquid injection method can be acquired by Eq. (1) (Nakamoto et al., 2006):

$$C = \frac{10 \times C_0 \times \rho \times V_{vol} \times R \times T}{M \times P_0 \times V_0} \quad (1)$$

where, C_0 = concentration of the liquid VOC (wt.%), ρ = density of the VOC (g mL^{-1}), V_{vol} = volume of liquid injected (μL), R = universal gas constant (L atm K^{-1} mol^{-1}), T = temperature inside VOC preparation chamber (K), M = molecular weight of analyte (g mol^{-1}), P_0 = pressure inside VOC preparation chamber (atm), and V_0 = volume of preparation chamber (L). The laboratory air was used as the reference gas to simulate real-world conditions.

2.4. Gas sensing characterization

The change in frequency of the sensors was monitored throughout the gas sensing cycle. The gas sensing cycle utilized for data