Bioelectronic Nose Using Odorant Binding Protein-Derived Peptide and Carbon Nanotube Field-Effect Transistor for the Assessment of Salmonella Contamination in Food

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Supporting Information

ABSTRACT: Salmonella infection is one of the major causes of food borne illnesses including fever, abdominal pain, diarrhea, and nausea. Thus, early detection of Salmonella contamination is important for our healthy life. Conventional detection methods for the food contamination have limitations in sensitivity and rapidity; thus, the early detection has been difficult. Herein, we developed a bioelectronic nose using a carbon nanotube (CNT) field-effect transistor (FET) functionalized with Drosophila odorant binding protein (OBP)-derived peptide for easy and rapid detection of Salmonella contamination in ham. 3-Methyl-1-butanol is known as a specific volatile organic compound, generated from the ham contaminated with Salmonella. We designed and synthesized the peptide based on the sequence of the Drosophila OBP, LUSH, which specifically binds to alcohols. The C-terminus of the synthetic peptide was modified with three phenylalanine residues and directly immobilized onto CNT channels using the π–π interaction. The p-type properties of FET were clearly maintained after the functionalization using the peptide. The biosensor detected 1 fM of 3-methyl-1-butanol with high selectivity and successfully assessed Salmonella contamination in ham. These results indicate that the bioelectronic nose can be used for the rapid detection of Salmonella contamination in food.

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odorants send electrical signals to higher regions of the brain, and finally, smell is perceived.\textsuperscript{21} LUSH is an OBP of Drosophila that specifically binds to alcohols.\textsuperscript{22} The structure of LUSH was defined, and the specific binding site for alcohol was previously reported.\textsuperscript{23} OBPs have been used for the development of biosensors which were combined with surface plasmon resonance,\textsuperscript{24} quartz crystal microbalance,\textsuperscript{25} surface acoustic wave,\textsuperscript{26−28} cantilever,\textsuperscript{29} electrochemical impedance spectroscopy,\textsuperscript{30,31} and FET.\textsuperscript{32} In this study, the peptide, which is derived from LUSH, was synthesized by the modification of the C-terminus with three additional phenylalanine (Phe) residues, and it includes specific binding site residues of LUSH. The synthesized peptide was directly immobilized onto CNT surfaces via the \(\pi-\pi\) stacking interactions between Phe residues and CNTs.\textsuperscript{33,34} The peptide-based bioelectronic nose that is inspired by the insect olfactory systems could detect 3-methyl-1-butanol with high specificity and selectivity. Furthermore, the bioelectronic nose could be used for the rapid assessment of \textit{Salmonella} contamination by simply diluting ham samples.

\section*{Experimental Section}

The CNT-FET was fabricated via the photolithography method as previously reported.\textsuperscript{35} Briefly, AZ5214 photoresist (PR) was used to fabricate a pattern on a SiO\(_2\) wafer. The wafer was dipped into octacyltrichlorosilane (OTS) solution (OTS/hexane = 1:500) for 7 min. The wafer was rinsed with hexane, acetone, and ethanol to remove the PR patterns. The OTS-patterned substrate was incubated in swCNT solution (0.02 mg/mL in 1,2-dichlorobenzene) for 30 s, and CNTs were specifically absorbed to the substrate. Afterward, Pd/Au (10/30 nm) electrodes were fabricated and passivated using PR to protect them from contacting the solution. The \textit{Drosophila} OBP-derived peptide (TKCVSLMAGTVNKKGEFFFF) was synthesized with purity higher than 90\% by Peptron (Korea). The peptide was diluted at 1 µg/mL in distilled water and stored at \(-20^\circ\text{C}\.\) For the immobilization of the peptide, 1.5 µL of peptide solution was placed on the CNT channel and incubated for 4 h at room temperature. After the immobilization process, unbound peptides were washed out 2−3 times with distilled water. An atomic force microscopy (AFM) system (MFP-3D, Asylum Research, USA) was used to analyze the immobilization of the peptide. The pristine CNT channel was imaged at a scan rate of 0.2 Hz. After the peptide immobilization, the CNT channel was imaged again at 0.05 Hz.

All the odorants were sequentially diluted to 1:10 using distilled water and stored at 4 \(^\circ\text{C}\) until used. Sliced ham (Korea) was purchased from a local market and stored at 4 \(^\circ\text{C}\). To prepare a \textit{Salmonella}-contaminated food sample, 4 \(\times\) 10\(^{10}\) CFU mL\(^{-1}\) of \textit{Salmonella typhimurium} LT2 was spiked on sliced ham samples, and then, the ham samples were stored at 25 \(^\circ\text{C}\) for 4 days.\textsuperscript{36} After the storage, 1 mL of distilled water was added to 1 mg of the food samples and diluted to 1:100 before use in the experiments.\textsuperscript{11} For measuring the current signal, 20 µL of distilled water was added on the CNT channel, and 2 µL of odorant or food samples was injected into the CNT channels using micropipettes. A bias voltage of 0.1 V was applied to the source and drain electrodes, and the gate voltage was grounded. Current signals were monitored using a 2326A source meter (Keithley, USA) and a MST 800 probe station (MS TECH, Korea). The bioelectronic sensors were used for only one measurement.

\section*{Results and Discussion}

For the detection of 3-methyl-1-butanol, which is an indicator of \textit{Salmonella} contamination, \textit{Drosophila} OBP-derived peptide (OBPP) has to be synthesized on the basis of the sequence and structure in the \textit{Drosophila} OBP, LUSH. The three-dimensional structure of the LUSH was previously identified.\textsuperscript{23} Thr57, Ser52, and Thr48 form a network of hydrogen bonds with alcohols providing a structural motif to increase binding affinity. Another research group also reported that Thr57 and Phe64 are essential amino acid residues that bind with ligands.\textsuperscript{25} The sequence of the synthesized peptide was TKCVSLMAGTVNKKGEFFFF. The three Phe residues were added to the C-terminus of the peptide for the immobilization onto CNTs via the \(\pi-\pi\) stacking interaction. Only the original part of the peptide is free, while the three Phe residues are immobilized on CNT. Therefore, it is considered that the Phe addition does not change the three-dimensional structure and the binding affinity of the peptide. Moreover, the peptide isoelectric point calculated with Compute pI/Mw\textsuperscript{37} was not changed and was maintained at 9.19 even after the addition of three Phe residues, since Phe is an uncharged residue. The peptide was directly immobilized on the CNT channel as a self-assembled monolayer, so that the field-effect can occur when the structure of the peptide is changed with the binding of 3-methyl-1-butanol (Figure 1). The AFM images of the CNT channel were measured before and after the immobilization of the peptide (Figure 2A). The height of the CNTs was scanned along the 5 µm region, and the average value of the height difference between two cases was calculated. The average height along the 5 µm region increased by 2.05 ± 2.01 nm (\(p\)-value 0.0079) (Figure 2B) after the immobilization process. Because the peptide length calculated with Hydrophathy plot\textsuperscript{38} was 2.19 nm, it is considered that the peptides were appropriately immobilized on the CNT channel.

To analyze the electrical characteristic of the biosensor, the drain-source current−voltage (\(I_{DS}-V_{DS}\)) curves of the CNT channel were measured before and after the immobilization of the peptide (Figure 3A). The resistance of the CNT channel was increased from 2.11 \(\times\) 10\(^6\) to 3.98 \(\times\) 10\(^8\) \(\Omega\), and the linearity of the \(I_{DS}-V_{DS}\) curve was maintained. When we increased the liquid gate voltage in distilled water, the \(p\)-type FET characteristic was observed even after the immobilization process showing the decrease in the current (\(I_{DS}\)) with increasing gate voltage (\(V_g\)) (Figure 3B). These results indicate that the peptide was successfully immobilized onto the CNT channel.
channel and the biosensor could be used for the detection of odorants.

The sensitivity and selectivity of the biosensor was demonstrated using 3-methyl-1-butanol, a standard odor that indicates Salmonella contamination in ham. Twenty μL of distilled water was placed on the CNT channel of the biosensor, and different concentrations of the odor solution were sequentially injected. Figure 4A shows the real-time conductance change with various concentrations of 3-methyl-1-butanol. The signal intensity is the difference between the final current value and the initial baseline, which is the initial current value at 0 M of 3-methyl-1-butanol. Prior to the injection of the distilled water, the diluent did not show significant change (data not shown), and no signal changes were observed at lower concentration than 1 fM. The biosensor exhibited the detection limit of 1 fM, and the signal increased with additional injection of higher concentrations. No significant signals were observed for the pristine CNT. The dose-dependent response of the biosensor was normalized by its maximum value and analyzed using the Langmuir isotherm model as previously reported (Figure 4B).11 The calculated equilibrium constant, $K_d$, was $5.25 \times 10^{13}$ M$^{-1}$. Previously, we have tested the sensor selectivity with different lengths of carbon chains.8,16 Herein, we focused on the functional group selectivity of the sensor, since LUSH is the OBP that specifically binds with alcohol, which has a hydroxyl group. Six control compounds, with chemical structures similar to 3-methyl-1-butanol, did not show any response for both the pristine CNT and the OBPP-functionalized CNT (Figure 5A). LUSH is the OBP that specifically binds to alcohol. OBPP is the synthetic peptide

Figure 2. Fabrication of the peptide-based CNT-FET. (A) AFM images and (B) height profiles of CNT channels before and after the immobilization of OBPP.

Figure 3. Electrical properties of the peptide-based CNT-FET. (A) Current–voltage curves and (B) liquid gate profiles of the CNT channels before and after the immobilization of OBPP.

Figure 4. (A) Real-time conductance changes to various concentrations of 3-methyl-1-butanol using the pristine and OBPP-functionalized CNT-FET. (B) Dose–response curves of the bioelectronic nose to 3-methyl-1-butanol. The signals were normalized by the maximum response of the biosensor ($10^{-8}$ M). Error bars, s.e.m. of three replicates.
LUSH is known as a Drosophila odorant binding protein to bind with alcohols. LUSH (Drosophila OBP)-derived peptide, containing essential amino acid residues for binding to alcohols, was synthesized with three additional phenylalanine residues at the C-terminus of the peptide and directly immobilized onto CNT channel of the biosensor via π−π interactions between Phe residues and CNTs. The peptide-based biosensor sensitively detected 3-methyl-1-butanol at a concentration of 1 fM and selectively distinguished the target odor molecule from other compounds with similar structures. Moreover, the biosensor detected the Salmonella-contaminated ham sample without a complex pretreatment process. Taken together, the bioelectronic nose, which relies on the peptide based on the insect OBP, can be used for the detection of Salmonella contamination in ham with a rapid and simple process. It is also expected that this approach can be applied to various food samples by using other OBPs or olfactory receptors which specifically bind with different food contaminators.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b03284.

Real-time response of pristine and OBPP-functionalized CNT-FET to inactivated Salmonella (Figure S1) (PDF)

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Notes

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