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A peptide receptor-based bioelectronic nose for the real-time determination of seafood quality

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ABSTRACT

We herein report a peptide receptor-based bioelectronic nose (PRBN) that can determine the quality of seafood in real-time through measuring the amount of trimethylamine (TMA) generated from spoiled seafood. The PRBN was developed using single walled-carbon nanotube field-effect transistors (SWNT-FETs) functionalized with olfactory receptor-derived peptides (ORPs) which can recognize TMA and it allowed us to sensitively and selectively detect TMA in real-time at concentrations as low as 10 fM. Utilizing these properties, we were able to not only determine the quality of three kinds of seafood (oyster, shrimp, and lobster), but were also able to distinguish spoiled seafood from other types of spoiled foods without any pretreatment processes. Especially, the use of small synthetic peptide rather than the whole protein allowed PRBNs to be simply manufactured through a single-step process and to be reused with high reproducibility due to no requirement of lipid bilayers. Furthermore, the PRBN was produced on a portable scale making it effectively useful for the food industry where the on-site measurement of seafood quality is required.

1. Introduction

Consumption of spoiled seafood can give rise to serious health problems, such as septicemia and gastroenteritis (Gram and Dalgaard, 2002; Shapiro et al., 1998). Therefore, studies to develop methods for the on-site determination of seafood quality have been increasing. Such an approach should allow for the sensitive and selective detection of volatile compounds generated from spoiled seafood. Many research groups have reported that trimethylamine (TMA) can be used as an effective indicator of seafood quality because its amount is increased by the decomposition of trimethyl-N-oxide in seafood after death (Dalgaard et al., 2006; Lone, 1992; Veciana-Nogués et al., 1997).

In order to detect TMA, analytical techniques such as gas chromatography–mass spectroscopy (GC–MS) (Milo and Grosch, 1995; Veciana-Nogués et al., 1996), ion mobility spectrometry (IMS) (Bota and Harrington, 2006), and high-performance liquid chromatography (HPLC) (Hyotylainen et al., 2001), have been typically used. Although these techniques have the advantage of precise quantitative analysis, they cannot be readily applied to on-site determination due to the requirements for complicated pretreatment processes, large instrumentations, and intricate detection methods. There have been significant advances in electronic (Adhoum et al., 2003; Alimelli et al., 2007; Natale et al., 2001; O'Connell et al., 2001; Pacquit et al., 2006) and bio-mimetic noses (Huang et al., 2011; Li et al., 1994; Mitsubayashi et al., 2004) that can be used for on-site determination; however, there are still many limitations of these approaches, such as sensitivity and selectivity. More recently, olfactory receptor (OR)-based bioelectronic noses have been demonstrated to be an effective solution to these limitations (Goldsmith et al., 2011; Kim et al., 2009; Lee et al., 2012b; Yoon et al., 2009). However, ORs require lipid bilayer membranes for their proper function; hence, manufacturing processes of OR-based sensors were complicate and labor intensive. Furthermore, because it is too difficult to thoroughly immobilize lipid bilayers on the chips, reusability and reproducibility could not be assured.

Herein, we describe a peptide receptor-based bioelectronic nose (PRBN) that can determine the quality of seafood in real-time through measuring the amount of trimethylamine (TMA) generated from spoiled seafood. PRBNs were manufactured using

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single walled-carbon nanotube field-effect transistors (SWNT-FETs) functionalized with olfactory receptor-derived peptides (ORPs) which can selectively discriminate TMA. Functionalization was performed through a single-step process using the property of SWNTs which aromatic rings are stacked on the surface by π–π interactions (Chen et al., 2001; Lee et al., 2012a). Additional phenylalanine (Phe) cluster was synthesized at the C-terminus of ORPs, which enabled us to simply cover the surface of SWNTs as a monolayer by a single-step process. The manufactured PRBN was able to sensitively and selectively detect TMA at a concentration of 10 fM and discriminate TMA from other similar molecules in real-time. Using a PRBN, TMA was successfully detected from three types of spoiled seafood (oyster, shrimp, and lobster) without any pretreatment processes. Also, PRBNs were capable of discriminating spoiled seafood from other types of spoiled foods, since TMA is specifically generated from seafood decomposition.

PRBNs were manufactured using small peptide rather than the whole protein, hence it provides a new insight in biological aspects and a huge potential for applications in industrial fields. Although the PRBN mimicked the olfactory reaction *via* membrane-translocated ORs, it was activated without lipid bilayers unlike the whole protein-based sensor due to the insignificance of tertiary structure. This results in increase in the capability for practical applications through improving reproducibility and reusability of the sensor as well as simplifying the manufacturing processes. We have demonstrated that it could be easily reused with a considerable reproducibility. Thus, PRBNs may be the most adjacent platform for practical uses. In addition, it was also produced on a portable scale making it effectively useful for the food industry where the on-site measurement of seafood quality is needed. Furthermore, PRBNs could be used in a wide range of different applications because TMA is also a regulated air-pollutant (Feldstein et al., 1974) and an indicator of diseases, such as bacterial vaginosis (Chaim et al., 2003; Wolrath et al., 2005) and trimethylaminuria (Chalmers et al., 2006).

2. Materials and methods

2.1. Preparation of peptides

ORP (NQLSNLSFSDLCFFF) and four kinds of fluorescein isothiocyanate (FITC)-conjugated peptides (GG, GGF, GGFF, and GGFFF) were synthesized by PEPTRON (www.peptron.com) with purity greater than 90%. The ORP were suspended at 1 mg/mL in DW. Four FITC-peptides were suspended at the same molar concentration (1 mM). The suspended peptides were kept frozen at −20 °C, and were melted right before they were used.

2.2. Preparation of odorants and food samples

100 μM solutions of TMA, triethylamine (TEA), dimethylamine (DMA), 2-methyl-1-propanol (MP), ethylacetate (EA), ethanol (EtOH), methanol (MeOH), and acetic acid (AA) (all from Sigma) in DW were first prepared and serially diluted to 1:10 using DW. The prepared odorant solutions were stored at 4 °C until they were used. For the preparation of spoiled food samples, oyster (South Korea), shrimp (Thailand), lobster (Canada), milk (South Korea), tomato (South Korea), broccoli (South Korea), and beef (South Korea) were purchased at a local market and subsequently ground. The samples were then stored in 15-mL Falcon tubes at 25 °C for different time periods. After the storage, 1 mL of DW was added into the Falcon tubes containing 1 mg of food samples, and the liquid fractions were diluted to 1:100 with DW before the experiments were performed.

2.3. Fabrication of PRBNs

A SWNT-FET was fabricated *via* the conventional photolithography method as previously reported (Lee et al., 2006). In brief, methyl-terminated octadecyltrichlorosilane (OTS) was patterned on a SiO_2 substrate *via* the photolithography method to create non-polar regions. The OTS-patterned substrate was then dipped into SWNT suspensions (0.05 mg/mL in dichlorobenzene) for approximately 10 s. During the dipping process, SWNTs were specifically adsorbed onto the exposed SiO_2 regions. Afterward, Ti (10 nm)/Au (20 nm) electrodes were fabricated *via* the photolithography process and covered with an insulating photoresist layer (DNR) to protect them from contacting the solution. The gap between the source and drain electrode was 6 μm. For the immobilization of ORPs, 1.5 μL of ORP-suspended DW solution was placed on the SWNT channel region of the fabricated chip for 4 h. During that period, the ORPs were coated on the SWNTs with a monolayer *via* self-assembly. Finally, the chips were washed with DW 3–4 times to remove unbound ORPs.

2.4. Characterization of peptide immobilization

The fluorescence, which was emitted from the four FITC-peptides immobilized on SWNT channels, was imaged using a fluorescence microscope (Oylmpus). The fluorescence intensities from the images were analyzed using ImageJ (NIH) and normalized by the maximum intensity. AFM imaging was performed under ambient conditions using the AFM system (MFP-3D, Asylum Research) in intermittent mode.

2.5. Electrical measurements for the detection of TMA

The electrical measurements were carried out using a probe station (MS tech) and a Keithley 2636A sourcemeter. In the experiments, 0.1 V was applied between the drain-source electrodes, whereas the gate voltage was grounded. 49.5 μL of a DW droplet was placed on the PRBNs, and 0.5 μL of the sample solution with specific target molecules was added. Afterward, the current changes between the drain and source electrodes were monitored.

3. Results and discussion

SWNT-based FET devices have high sensitivity to specific analytes with detection limits as low as the pico- or femto-molar range. Thus, they have been used in various applications by many groups. In most cases, biomolecules, such as enzymes (Zhang et al., 2004), receptors (Kim et al., 2011; Sánchez-Acevedo et al., 2009), antibodies (Kim et al., 2008), and aptamers (Maehashi et al., 2006), were immobilized on the surface of SWNTs to improve the selectivity of FET sensors. For the detection of TMA which emits an irritating and ammonia-like odor, ORP (NQLSNLSFSDLC) can be used (Wu and Lo, 2000). ORP is a mutated α-helix peptide sorted out from a natural olfactory receptor that can specifically recognize TMA. In order to immobilize the ORPs, we employed an interesting property of SWNTs, which allows specific molecules with extensive [pi]-systems to be stacked on the wall through π–π interactions. Thus, we hypothesized that SWNTs could be non-covalently coated with specific ORP containing phenylalanines, a natural amino acid with π-systems as depicted in Fig. 1. In this article, we aimed to exploit the biological analyte-selection ability in the development of a novel electronic sensor by conjugating ORPs onto SWNT-FETs through a single-step process.