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研究課題名
昆虫嗅覚センサー情報処理による匂い源探索装置の開発

Title
Development of odorant detection system based on insect olfactory sensor

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研究代表者：東京大学先端科学技術研究センター、教授、神崎亮平

Ryohei Kanzaki
Research Center for Advanced Science and Technology, The University of Tokyo,
Professor,

Summary

Sensitive and real-time odorant sensing technology is demanded for a safety and security in our life and an improvement of the life quality, including not only food administration, water control, and monitoring environment of agricultural products but also risk management; i.e. detection of explosive and drugs in airport; monitoring pollutants in the environment, and disease diagnosis by the odor. Current odorant sensors based on metal-oxide semiconductor devices, quartz crystal microbalances, or surface acoustic waves have been used for practical odorant-detection applications. However, there are some shortages in these odorant sensors. For example, the lack of the coating materials limits the number of target odorants in the metal oxide semiconductor sensors. The odorant sensor using quart crystal microbalances does not have enough sensitivity due to the disturbance by humidity. Currently, in order to overcome these disadvantages, it is required to develop odorant sensors based on innovative sensing technology that detect various types of odorants with high sensitivity and selectivity in real-time.

Living organisms, especially insects, are equipped with sophisticated olfactory mechanism to enable them to selectively and sensitively detect various types of odorants. It was reported that this odorant detection in insects is mainly achieved by odorant receptors that function in their antennae. Therefore, in this study, focusing on the insect odorant receptors, we aim to develop odorant sensors that allow detection of various types of odorant with higher performances.

First, in order to create a basic technology for development of odorant sensor elements based on Sf21 cell lines expressing insect odorant receptors, we established the cell lines expressing pheromone receptors from the silkmoth, *Bombyx mori*, to evaluate the performances as odorant sensor elements. The cell lines co-expressing pheromone receptor and co-receptor along with fluorescent indicator protein, GCaMP3, detect corresponding pheromone components by increasing their fluorescence intensities. The cell lines exhibited dose-dependent responses to target components with the detection limit of 300 nM (several tens parts per billion (ppb) in solution) and selective responses in accordance with the selectivity intrinsic to the expressed receptors. In addition, the Sf21 cell lines were available for detecting pheromone components with invariable responsiveness over at least 2 months, thereby overcoming the short life span of biosensors for practical use. From these results, we established the methodology for developing novel cell-based odorant sensor elements based on Sf21 cell lines expressing insect odorant receptors.

Next, by using the same method as that used for creating the cell lines expressing pheromone receptors, we established Sf21 cell lines expressing general odorant receptors from the fruit fly, *Drosophila melanogaster*, for application to general odorants. The established cell lines were

found to detect general odorants by displaying fluorescent intensity increase. For the development of odorant sensor elements with higher sensitivity, we established a methodology to create cell lines that detected general odorant at the scale of ppb in solution by improving expression vectors of odorant receptor genes and changing the types of fluorescence indicator proteins. Using this method, we succeeded to create cell lines that detect mold odorants, geosmin and 1-octen-3-ol with detection limit of 300 nM (several ten ppb in solution). The cell lines could detect geosmin even in the presence of background odor derived from foods or beverages, indicating that these cell lines were available as practical odorant sensor elements. From these results, we demonstrated that our methodology is applicable for developing practical cell-based odorant sensor elements that detect various types of odorants from pheromones to general odorants with high sensitivity and selectivity.

As odor is composed of various types of odorants, odorant sensor needs to be developed by acquiring the signals from array of odorant sensor elements with different odorant selectivity for discriminating the odor. Finally, we tried to establish two methodologies for arraying several cell lines by using microfabrication technique and cell patterning technique. For the microfabrication techniques, we designed and fabricated polydimethylsiloxane (PDMS)-glass chip with two microfluidic channels, into which two cell lines were separately integrated. The chip arraying two cell lines, each of which responds to a specific odorant, succeeded to discriminate two odorants by showing the patterns of fluorescence intensity change. For the cell patterning technique, we established a methodology for arraying four cell lines onto a glass surface by using biocompatible anchor for membrane (BAM) and PDMS sheet. By arraying four cell lines having different odorant selectivity from each other, we succeeded to develop an odorant sensor array that discriminated four odorants as the fluorescence intensity pattern.

Through these studies, we demonstrated that the use of insect odorant receptors enables us to develop odorant sensor that combines both high sensitivity and high selectivity. In addition, we successfully established innovative platform to develop a novel odorant sensor with high performances exceeding existing odorant sensors.