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Odor Quantification of Aromatic Alcohols Using Artificial Olfactory Epithelium

Ryosuke Izumi*, Hiroshi Abe, Kenshi Hayashi and Kiyoshi Toko

Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

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The quantification of the senses requires the interpretation of a biological recognition process. In olfactory senses, the receptors on the olfactory epithelium receive odor molecules by recognizing not their whole rigid chemical structures but their common properties. In this study, we attempted to develop an artificial olfactory epithelium system that can recognize the molecular information of odor. This system was constructed using an electrochemical cell and has been developed by the functionalization of sensor channels with the composition of electrolyte solution and with surface modification technologies. As a result, the odor information of aromatic alcohols could be represented, depending on molecular substructures.

1. Introduction

The quantification of five senses, visual, auditory, tactile, gustatory and olfactory senses, requires the interpretation of a biological recognition process. In the visual sense, receptors recognize colors and light intensity depending on the difference in wavelength.[1] In the auditory sense, the difference in sound frequency is selectively recognized, depending on the intrinsic electrical properties of hair cells.[2,3] As just described, what the receptors of physical senses recognize depends on physical quantities; therefore, these quantities can be suitable for the quantification of human senses.

In the gustatory sense, which is one of the chemical senses, it is noted that there are five fundamental tastes: sourness, sweetness, bitterness, saltiness and umami taste. These tastes are the information that human can feel psychologically, so it is necessary to associate the physical quantities obtained by physicochemical detection methods with them. To quantify them, a taste-sensing system (SA402B, Intelligent Sensor Technology)

*Corresponding author: e-mail: izumi@belab.ed.kyushu-u.ac.jp

is developed and achieved by quantifying them by measuring the electric potential change of lipid membranes.[4] On the other hand, there is no such a fundamental odor in the olfactory sense, and hence, it is difficult to quantify the odor depending on human perception. Therefore, it requires knowledge of a biological olfactory recognition process.

In a biological olfactory system, odor molecules are received by about 350 odor receptors on the olfactory epithelium. First, the odor molecules are dissolved into the nasal mucus, and then, they are received by the odor receptors by recognizing not the whole rigid chemical structures of the odor molecules but their common properties. This is referred to as the odotope hypothesis. Subsequently, the detection of the odor molecules activates the odor receptors, and then, electrical signals are transported from the receptors to the brain. In short, odor is determined by a combination of the activated receptors, which is called an odor code.[5,6] This recognition system is similar to the gustatory system; thus, molecular information can be thought as an indicator of odor quantity.

The aim of this study is to develop an artificial olfactory epithelium and a sensing surface recognizing the molecular information. In the development of the system, we paid attention to substructures as detection targets to recognize the molecular information of the odor. In this study, we attempt to quantify the odor on the basis of molecular substructures.

2. Experiment

2.1 *Artificial olfactory epithelium chip*

Figure 1 shows the artificial olfactory epithelium chip developed here. This chip was formed by evaporating gold onto a glass substrate and by creating the pattern of an electrode structure using a photolithography technique. It is composed of four channel sensors; each sensor has a pair of two electrodes. This multichannel chip was used to simultaneously measure odor molecules with several channels having different response properties. In this measurement, a water membrane covered the electrodes of each channel and trapped the odor molecules in gas. The water membrane is a mixture of 40% 100 mM KCl and 60% glycerin, which keeps the water membrane thickness constant for a long time. The water membrane was formed by immersing the chip into the solution and tore off the spin coater at a speed of 2500 rounds per minute. Consequently, the thickness of the water membrane is very small as well as that of the biological nasal mucus, which is approximately 10 μm.

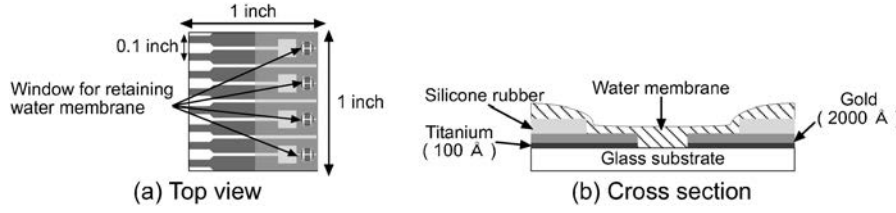


Fig. 1. Artificial olfactory epithelium chip.

2.2 *Multichannel electrochemical impedance measurement*

In this system, we measured an electrode impedance spectrum. An artificial olfactory epithelium chip was connected to a current detection resistance of 1 MΩ in series; then, a sine wave electric potential of 0.1 V amplitude from a frequency response analyzer (FRA) (NF5020, NF Electronic Instrument) was applied to the circuit. Odor gases were introduced into the chamber where a sensor was set with a standard gas generator (PD-1B, Gastec). As the water membrane is very thin, the electrode impedance was easily affected by environmental changes, such as the temperature, humidity and switching flow path of the odor gas. The odor gas, therefore, was produced at the controlled temperature, by flowing the air through a water bath and by setting the path of the odor sample to be the same as that of the odorless gas.

In previous studies, the electrode impedance was measured with a cyclically controlled potential.[7,8] Although various information on the interaction between the electrode surface and the odor molecules can easily be detected, it took a long time to stabilize the electrode impedance, because the electric double layer on the electrode surface was easily changed by potential control. Moreover, as the electric potential was applied for a long time, the electrode was damaged easily. On the other hand, the electrode impedance can be stabilized quickly, reducing the damage to the electrode in the system of this study. Moreover, it is easy to measure the impedance of multiple channels simultaneously. The multichannel sensor, however, requires several detectors with specificity to peculiar molecular substructures in this system. In this case, we paid attention to aromatic alcohol, where odor quantification was made on the basis of a hydroxyl group and an aromatic ring.

2.3 *Functionalization of artificial olfactory epithelium*

2.3.1 *Water membrane with cellulose phosphate (P cellulose)*

A high specificity to a hydroxyl group was previously achieved by adding cellulose phosphate (P cellulose) into the water membrane.[8] The addition of P cellulose, a cation-exchange resin, might affect the interaction between the electrode surface and the odor molecules through a change in the pH of the water membrane. Therefore, this membrane was also utilized in this system.

2.3.2 *Hydrophobic and hydrophilic benzene-patterned self-assembled monolayers (SAMs)*

To detect an aromatic ring with a high specificity, we developed a benzene-patterned self-assembled monolayer (SAM).[8–10] Since the electrode surface became hydrophobic by SAM modification, the affinity to a hydrophobic aromatic ring might be improved. This benzene-patterned SAM was obtained by immersing the electrode into 100 μM 1-octanethiol solution diluted with benzene and removing the adsorbed benzene by ethanol rinse. By surface analysis, the gold surface was determined to be covered by a hydrophobic membrane and to have a high inhomogeneity and roughness.[11] Therefore, it is considered that the benzene-patterned SAM was formed similarly to that in Fig. 2(a). In this case, we call this SAM a hydrophobic benzene-patterned SAM.

However, the utilization of the hydrophobic benzene-patterned SAM can involve a

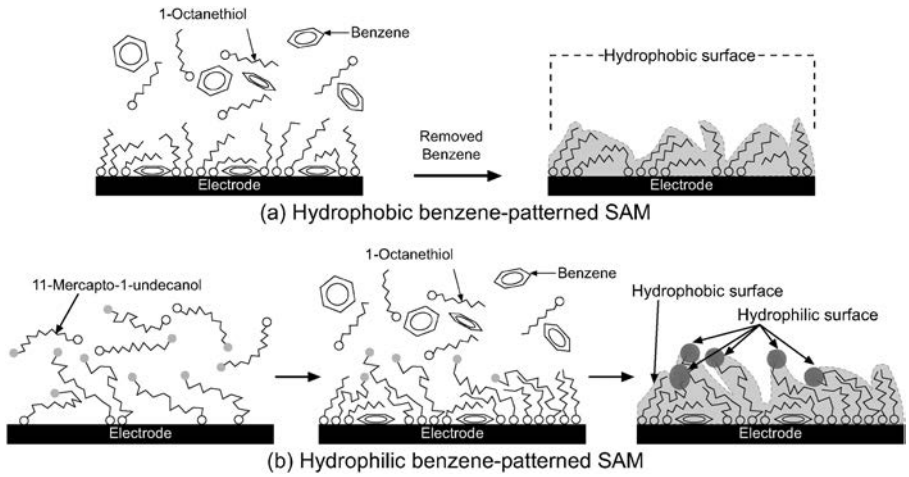


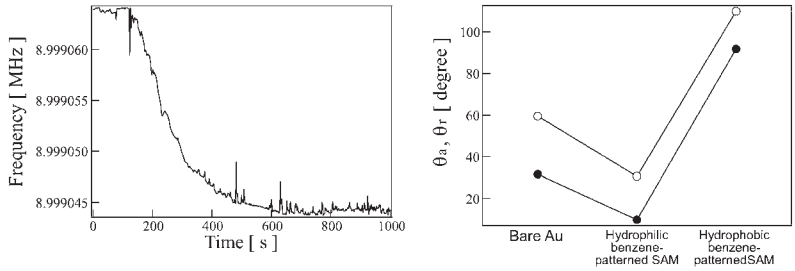
Fig. 2. Hydrophilic and hydrophobic benzene-patterned SAMs.

difficulty in holding the water membrane on such a hydrophobic surface. Therefore, we attempted to mix thiols with a hydrophilic group at a terminal group into the hydrophobic benzene-patterned SAM; then, we fabricated the same surface structure as that in Fig. 2(b). In this study, 11-mercapto-1-undecanol (HS-C11-OH) was utilized as a hydrophilic SAM.

To determine fabrication conditions, we performed the surface analysis of the hydrophilic benzene-patterned SAM. Figure 3(a) shows the temporal change in frequency due to the adsorption of HS-C11-OH onto the gold surface of quartz-crystal microbalance (QCM) (UQ200, U. S. I. System). The adsorption of HS-C11-OH was induced by immersing the electrode into 2 mM HS-C11-OH solution diluted with ethanol. From the obtained result, HS-C11-OH was observed to be completely adsorbed onto the gold surface and the amount of HS-C11-OH adsorbed was estimated to be 21.39 ng. To fabricate a rough benzene-patterned SAM membrane, it is preferred that the amount of SAM adsorbed is small. Therefore, the time of immersion into HS-C11-OH solution was set at 1 min, and the amount of HS-C11-OH adsorbed onto the surface was 5.95 ng. The thickness of the membrane was approximately 1.26 nm.

Furthermore, the contact angle of the hydrophilic benzene-patterned SAM was measured in order to confirm the hydrophilicity of the surface shown in Fig. 3(b). In Fig. 3(b), θ_a represents the aggressive contact angle, and θ_r represents the regressive contact angle; these parameters indicate the hydrophilicity/hydrophobicity of the surface. In addition, $\Delta\theta$ is the difference between θ_a and θ_r and stands for the roughness of the surface.

From the figure, the contact angle of the benzene-patterned SAM with HS-C11-OH is smaller than 90°; thus, the surface can be considered hydrophilic. Therefore, this surface is sufficiently hydrophilic to retain the water membrane.



(a) Adsorbed amount of HS-C11-OH using quartz-crytal microbalance

(b) Contact angle of hydrophilic/ hydrophobic benzene-patterned SAM

Fig. 3. Surface analysis results for hydrophilic benzen-patterned SAM.

Since the hydrophobic benzene-patterned SAM cannot retain the water membrane on the surface, we could expect that the measurement with it is impossible. However, its electrode impedance was measured when the artificial olfactory epithelium chip was washed with the alkali solution (10 mM KOH) as a pretreatment. As the conduit between the gold electrodes became hydrophobic by this washing, this conduit could retain the water membrane. Therefore, the impedance of the electrode edge could be measured; thus, the measurement with the hydrophobic benzene-patterned SAM became possible. As a result, we utilized both hydrophilic and the hydrophobic benzene-patterned SAMs to collect more information.

2.4 *Measured odorants*

In this study, the odorants shown in Fig. 4 were measured. This measurement is performed to quantify aromatic alcohols using molecular information with four channels, i.e., a bare-Au channel, the water membrane with P cellulose, and the hydrophobic and hydrophilic benzene-patterned SAMs.

3. Results and Discussion

Figure 5 shows the responses to alcohols with the bare-Au channel. This figure indicates the temporal change in the change ratio of the electrode impedance ($\Delta Z_e/Z_0$) by flowing the odor gas. Figure 5(a) shows the real part of $\Delta Z_e/Z_0$, $\Delta R_e/R_0$ and Fig. 5(b) shows the imaginary part of $\Delta Z_e/Z_0$, $\Delta X_e/X_0$.

From the figure, the electrode impedance decreased by flowing the odor gas. A transient temporal peak appeared in each response at around 400 s after the start of odor gas flow. This peak can be seen in all responses; therefore, this peak may reflect the influence of the switching flow path of the odor gas. As a result, we selected $\Delta Z_e/Z_0$ around 1000 s as the response to the odor molecules. Figure 6(a) shows the profiles of the $\Delta R_e/R_0$ and $\Delta X_e/X_0$ responses to the odor molecules measured with each channel. Moreover, the response data was standardized for each odor molecule, in order to avoid

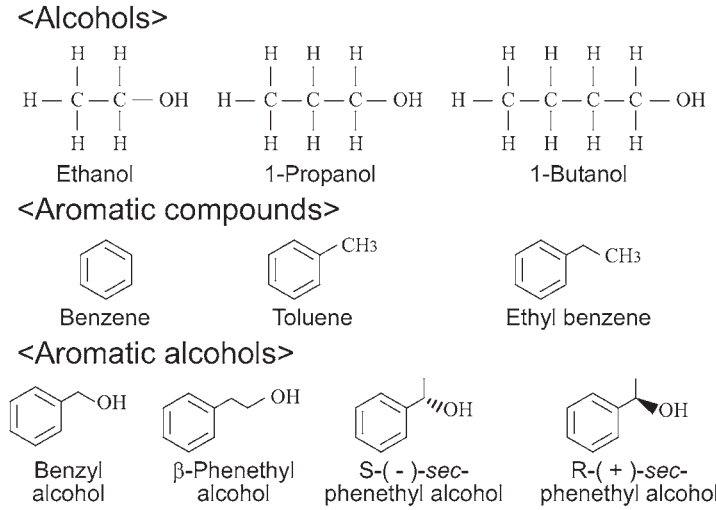
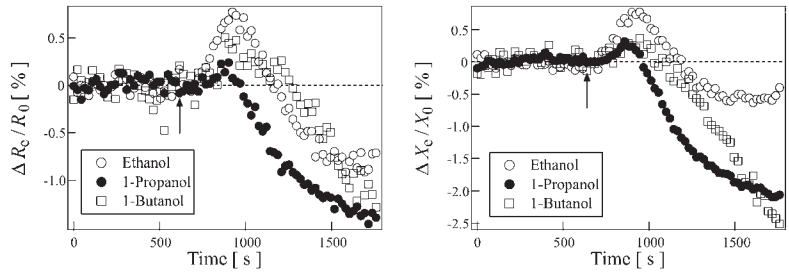


Fig. 4. Measured odorants.

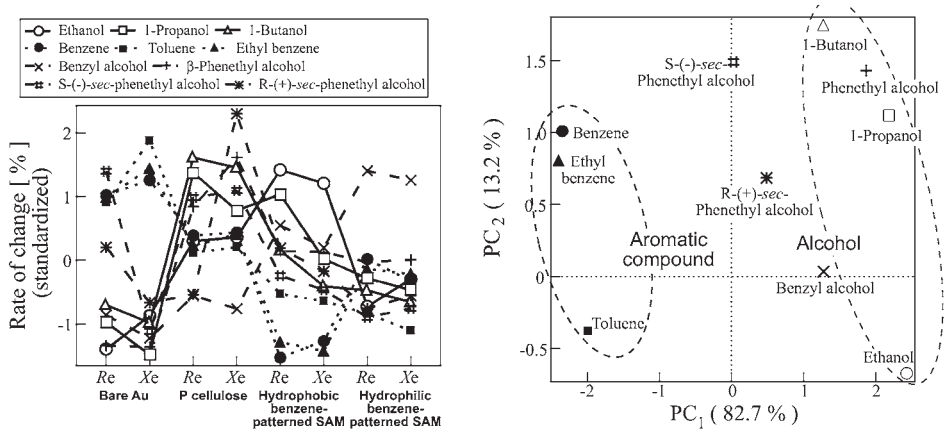


(a) $\Delta R_e / R_0$ (b) $\Delta X_e / X_0$

Fig. 5. Responses to alcohols with bare-Au channel. Each odor gas was started to flow into the sensor cell at the arrow point.

the influence of the difference in odor strength depending on the concentration or the vapor pressure of the odorants. In addition, Fig. 6(b) shows the principal component diagram of the result in Fig. 6(a).

In Fig. 6(a), alcohols show different responses with the bare Au and hydrophobic benzene-patterned SAM from aromatic compounds. In addition, the response profiles of alcohols, especially 1-propanol and 1-butanol, are very similar, and those of aromatic compounds are quite similar. Furthermore, alcohols and aromatic compounds are plotted separately at PC_1 in Fig. 6(b). According to the contribution ratio, alcohols and aromatic compounds are distinguished well at the $\Delta R_e/R_0$ and $\Delta X_e/X_0$ of the bare-Au and hydrophobic benzene patterned SAM channels. It might be considered that the bare-Au



(a) Channel response to odor molecules (b) Principal component diagram of the response

Fig. 6. Response to odor molecules: (a) channel response and (b) principal component analysis.

channel reflects the adsorption affinity of the odor molecules to the Au surface and that the hydrophobic benzene-patterned SAM channel reflects the affinity of the hydrophobic interaction between the hydrophobic surface and the odor molecules. In this manner, it can be considered that this sensor can distinguish odor depending on molecular substructures. Thus, the tendency of response to aromatic alcohols varies with the structures. If the odor of aromatic alcohols is contributed only by hydroxyl group and aromatic ring, the odor of the aromatic alcohols expressed by aromatic/alcoholic strength can be quantified with the first principal component score, as shown in Table 1.

From Table 1, it can be considered that the odor of the aromatic alcohols measured is mainly contributed by the hydroxyl group. By comparing the aromatic alcohols, the hydroxyl group was determined to mainly contribute to the odors of benzyl alcohol and β-phenethyl alcohol. β-Phenethyl alcohol has the largest distance between the hydroxyl group and the aromatic ring. If the distance is large, it can be considered that the property of the hydroxyl group can likely be reflected by the molecular information. Otherwise, the difference among benzyl alcohol, S-(-)-sec-phenethyl alcohol and R-(+)-sec-phenethyl alcohol is the chemical bond beween the hydroxyl group and the carbon atom; therefore, these results might reflect this difference. From the psychological viewpoint, benzyl alcohol and β-phenethyl alcohol are known as the components of perfumes, and S-(-)-sec-phenethyl alcohol and R-(+)-sec-phenethyl alcohol have a thinner smell. Moreover, R-(+)-sec-phenethyl alcohol can smell like alcohol rather than S-(-)-sec-phenethyl alcohol. In this manner, these odors deffer with human perception.

In the previous studies, the results indicated that the odors of aromatic alcohols are mainly dominated by the aromatic ring.[7,8,12] It can be considered that the aromatic ring has a stronger adsorption affinity to the surface than the hydroxyl group. From the psychological viewpoint, the odor of β-phenethyl alcohol is very different from that of

Table 1
Odor quantification of aromatic alcohols based on PC_1

	Benzyl alcohol	β-Phenethyl alcohol	S-(-)-sec-phenethyl alcohol	R-(+)-sec-phenethyl alcohol
Alcoholic strength (%)	76.2	88.4	50.6	60.0
Aromatic strength (%)	26.8	11.6	49.4	40.0

ethyl benzene, although the difference in their chemical structure depends only whether there is a hydroxyl group or not. In spite of this, the measurement in this study yielded less information from each channel owing to the abscence of potential control, and the influence of the hydroxyl group was reflected by the results well. As PC_1 was largely contributed by the hydrophobic benzene-patterned SAM channel, which might indicate the hydrophobic interaction between the electrode surface and the odor molecules, the difference between the hydrophilic hydroxyl group and the hydrophobic aromatic ring can easily be reflected. In contrast, the contribution rates of the P cellulose and hydrophilic benzene-patterned SAM channels to PC_1 were small, although they were developed for the detection of the hydroxyl group. However, in the case of the P cellulose channel, the difference between the hydroxyl group and the aromatic ring was sufficiently distinguished when we evaluated the differences between the responses of the P cellulose channel and those of the bare Au channel, rather than when we evaluated the differences with only those of the bare-Au channel. In addition, in the case of the hydrophilic benzene-patterned SAM channel, the hydroxyl group and aromatic ring were also distinguished with its responses. In this manner, the difference of the odor depending on the substructures of the odor molecules can be detected more efficiently. Furthermore, this measurement takes a shorter time than the previous measurement; therefore, it can be considered that the utilization of this measurement leads to more efficient odor measurement.

4. Conclusion

In this study, an odor-sensing system, i.e., an artificial olfactory epithelium, was developed by imitating biological olfactory systems. It could detect molecular information by recognizing molecular substructures, and the odor of aromatic alcohols could be evaluated using the molecular information. These results indicate the potential of the system for odor quantification. In addition, if odor information can be digitized in this manner, the digitized data will be applicable in various areas, such as environment monitoring, the odor evaluation of food and odor reproduction.

References

1 J. Nathans, D. Thomas and D. Hogness: Science **232** (1986) 193.
2 U. Pirvola, J. Ylikoski, R. Trokovic, J. M. Hebert, S. K. McConnell and J. Partanen: Neuron **35** (2002) 671.
3 J. Zuo: J. Neurobiol. **53** (2002) 286.
4 K. Toko: Biomimetic Sensor Technology (Cambridge University Press, 2000) Chap. 6.
5 L. Buck and R. Axel: Cell **65** (1991) 175.
6 R. C. Araneda, A. D. Kini and S. Firestein: Nature Neuroscience **3** (2000) 1248.
7 R. Izumi, S. Etoh, K. Hayashi and K. Toko: Proc. Int. Symposium on Olfaction and Electronic Nose-ISOEN'05 (Barcelona, 2005) p. 280.
8 R. Izumi, S. Etoh, K. Hayashi and K. Toko: Tech. Digest Transducers'05 (Seoul, 2005) p.1884.
9 K. Hayama, K. Hayashi and K. Toko: Sens. Materials **15** (2003) 403.
10 K. Hayashi, P. Ivarsson, S. Michiwaki, K. Masunaga, R. Izumi and K. Toko: Proc. 22nd Sensor Symposium, (Tokyo, 2005) p. 285.
11 K. Masunaga, S. Michiwaki, R. Izumi, P. Ivarsson, F. Bjorefors, I. Lundstrom, K. Hayashi and K. Toko: Proc. International Meeting on Chemical Sensors (Brescia, 2006) TO3.3.3 #228.
12 R. Izumi, K. Hayashi and K. Toko: Sensors and Actuators B **99** (2004) 315.