Detection of Sugars Using Lipid/Polymer Membranes

Masaaki Habara*, Daiki Beppu[1], Hong Cui,
Hidekazu Ikezaki[2] and Kiyoshi Toko[1]

Graduate School of System Life Sciences, Kyushu University, Fukuoka 819-0395, Japan
[1]Graduate School of Information Science and Electrical Engineering, Kyushu University,
Fukuoka 819-0395, Japan
[2]Intelligent Sensor Technology, Inc., 1800 Onna, Atsugi, Kanagawa 243-0032, Japan

Lipid/polymer membranes were modified with a phenolic compound, gallic acid, to obtain a high sensitivity to sugars, such as sucrose, glucose and fructose. Electric responses to sugars were measured with surface-modified membranes composed of tetradodecylammonium bromide, di-n-octylphenylphosphonate and polyvinyl chloride. The spectroscopic properties of gallic acid in an aqueous solution and in the presence of amphiphilic molecules in a buffer solution were studied. The obtained results show that the change in the absorption spectrum of gallic acid is pH- and time-dependent, and is susceptible to electric responses to sugars.

1.   Introduction

A taste sensor with lipid/polymer membranes has been developed as a biomimetic sensor.[1] The sensor transduces information of taste into electric signals with a potentiometric measurement. Taste qualities are divided into five basic tastes: saltiness, sourness, bitterness, umami taste and sweetness. The taste sensor is designed to detect each basic taste selectively. The sensor output is recognized as unique patterns for chemical substances with different taste qualities, while it shows similar patterns for chemical substances with similar tastes.

Sweet taste substances, such as sucrose, fructose and glucose, are carbohydrates with an aldehyde group or ketone group, or their derivatives. Therefore, a potentiometric measurement with lipid/polymer membranes shows a low sensitivity to sugars compared with other basic taste substances.

---

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

In our previous study, we developed sweet-sensitive lipid/polymer membranes composed of phosphoric acid di-n-hexadecyl ester, tetradodecylammonium bromide, di-n-octylphenylphosphonate and polyvinyl chloride.[2–4] Although the sensor responded to sucrose at a concentration as low as 100 mM, the sensitivity was not sufficient for evaluating sweetness effectively.

In this study, the modification of a surface with phenolic compounds was performed to enhance sensitivity to sugars. Phenolic compounds contain more than one phenolic hydroxyl group and precipitate biological materials, such as protein. These organic compounds consist of gallic acid derivatives. Gallic acid is esterified to polyol groups, such as glucose, or to galloyl groups to yield more complex polyphenols.[5] It is also suggested that gallic acid shifts the pKa value in the presence of amphiphilic molecules in the buffer solution with UV spectrum measurements.[6] We fabricated a membrane composed of cationic lipids and tetradodecylammonium bromide, and modified the membrane surface with gallic acid to obtain a high sensitivity to sugars.

2. Materials and Methods

Figure 1 shows the schematic illustration of a taste sensor composed of a sensing element, a controller and a date-processing terminal.[1] In the sensing element, the membrane potential between the working electrode and the reference electrode was measured. After the performance of analog-to-digital conversion, signals were transmitted into a computer.

The transducers of the taste sensor are composed of lipids immobilized with polyvinyl chloride.[1,7,8] Figure 2 shows the chemical structures of membrane-forming materials and gallic acid. Tetradodecylammonium bromide (TDAB) was mixed in a test tube
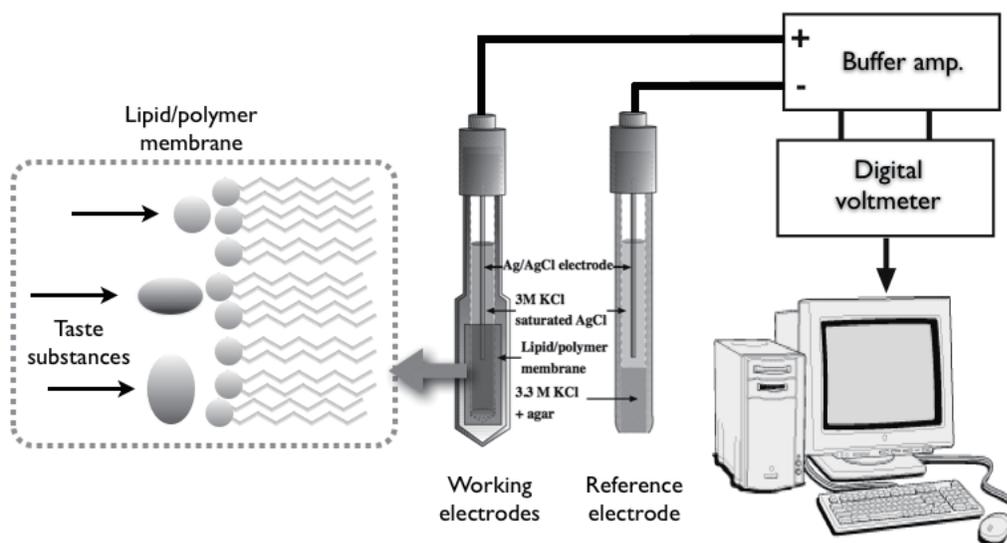

Fig. 1. Schematic diagram of taste-sensing system SA402B (Intelligent Sensor Technology, Inc.).
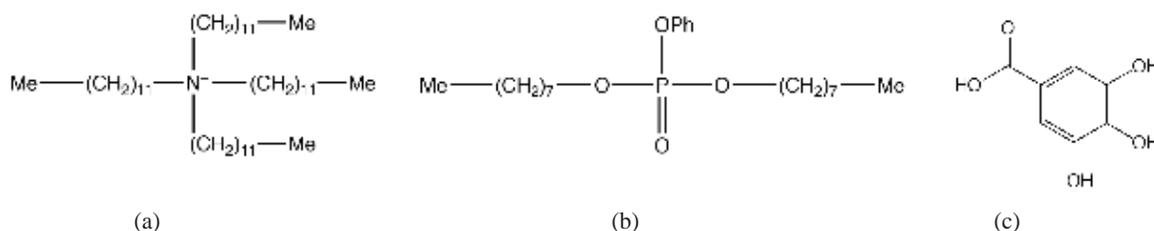
Fig. 2. Chemical structures of (a) tetradodecylammonium bromide (TDAB), (b) di-n-octylphenylphosphonate (DOPP) and (c) gallic acid.

with 800 mg of polyvinyl chloride (PVC) and a plasticizer, di-n-octylphenylphosphonate (DOPP) dissolved in tetrahydrofuran. Then, the mixture was dried on a glass plate.

   The membrane was preconditioned by the following procedures; it was immersed into 0.05% (wt/wt) gallic acid solution for 48 h at RT and then into 10 mM KOH + 30% EtOH + 100 mM KCl solution for 15 min.

   The sensor output is the difference in potential between the control and sample solutions. The electric response obtained from the control solution is defined as the reference potential Vc. Consecutively, the electric potential of the sample solution Vs was measured. The control solution is composed of 30 mM potassium chloride and 0.3 mM tartaric acid, which is almost tasteless and is used as solvent. The difference in potential between Vs and Vc provides information on taste. After the measurement, cleaning was performed to remove adsorbed tastants and refresh the membrane surface using 10 mM KOH + 30% EtOH + 100 mM KCl solution. The series of measurements was typically repeated five times for each sample.

   UV spectra were recorded using UV spectrophotometer UV-3100 (Shimazu, Japan) with quartz cuvettes. The pH was adjusted to 2 and 3 by adding appropriate volumes of 0.5 N HCl to the aqueous sample, and to 12 by adding 1 N potassium hydroxide solution.

3.   Results and Discussion

   Figure 3 shows electric responses to 1 M sucrose solution. The surface of lipid/polymer membranes was modified with gallic acid. The amount of the cationic lipid TDAB changes the membrane potential and surface hydrophobicity. The electric density and hydrophobic property are susceptible to the adsorption of gallic acid on the membrane surface. The concentration of DOPP also affects the membrane potential.[9] From these results, the potential peaks of the sucrose sample exist between 2.25 and 2.5 mg of TDAB and 1.5 ml of DOPP with 800 mg of PVC.

   Figure 4 shows the dependences of the electric responses on the concentrations of sucrose, glucose and fructose. The sensor is sensitive to each sugar at a concentration as low as 100 mM, which corresponds to our discrimination threshold for sucrose. From Figs. 3 and 4, the surface-modified lipid membranes could induce membrane potential changes.
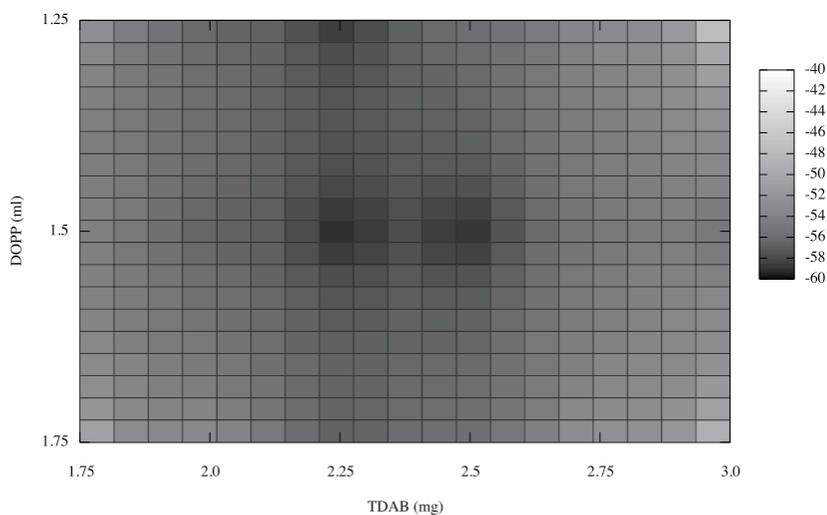
Fig. 3.   Electric responses to 1 M sucrose solution. Potential peaks are located between 2.25 and 2.5 mg of TDAB and 1.5 ml of DOPP with 800 mg of PVC.
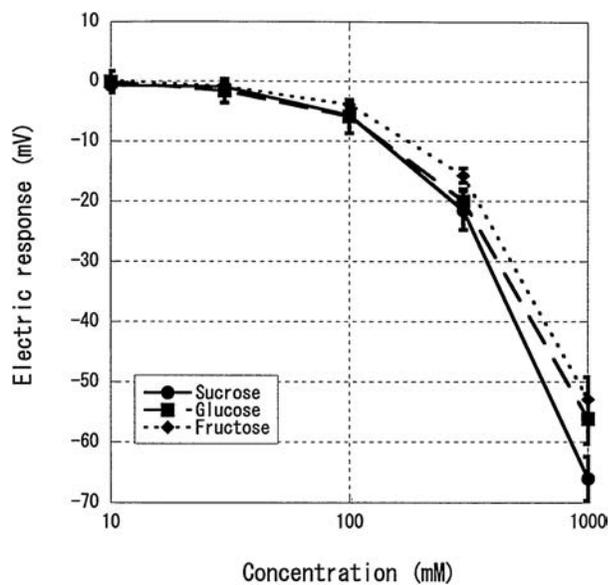

Fig. 4.   Electric response dependences for 1 M sucrose, glucose and fructose with sweet-sensitive lipid/polymer membrane.

Gallic acid shows two peaks in the absorption spectrum of the buffer solution (213 and 270 nm). As shown in Fig. 5, the spectrum of gallic acid was changed with pH 12 solution because of the OH group on the benzen ring of gallic acid. The spectral changes
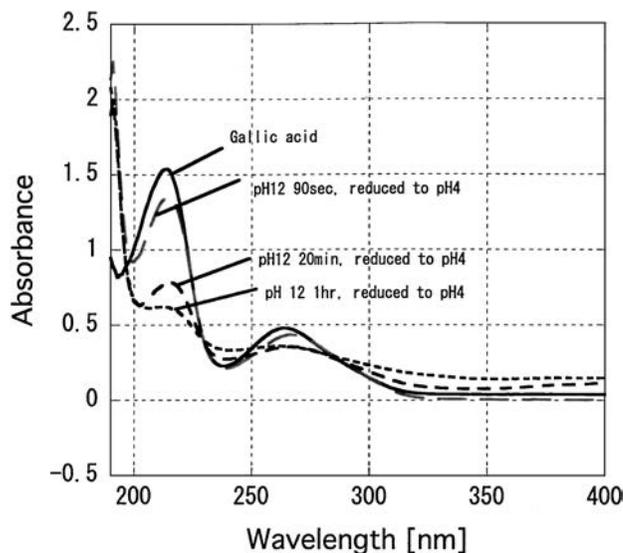
Fig. 5.   Reversibility of absorption spectra of gallic acid (5×10^{-5} M) after reducing pH of solution from 12 to 4.

and reversibility are time-dependent. Changing the pH of the buffer solution from 12 to 4 does not regenerate the original spectrum, depending on immersion time in the pH 12 solution. It is suggested that the number and location of phenolic OH groups are primarily responsible for the spectral changes.[10]

Figure 6 shows the corresponding results obtained from the taste sensor system SA402B. The electric responses to 1 M sucrose solution were measured under the following conditions; the membranes were absorbed into 0.05% (wt/wt) gallic acid solution for 48 h at RT and then immersed into 10 mM KOH + 30% EtOH + 100 mM KCl solution for 15 min. The membranes were immersed into the pH 12 solution. After 90 s, 20 min and 1 h, pH was reduced to 4. As shown in Fig. 6, the electric responses to sucrose were reduced, depending on the immersion time of the pH 12 solution.

The absorption spectra of gallic acid with lipids in the pH range 2–7 are illustrated in Fig. 7. The pH of the buffer solution is 12 for 90 s; this pH was reduced to 4 for 120 s, which was repeated six times. The calculated pKa of gallic acid is 4.34 compared with the pKa of 4.27 reported in the literature.[11] In the presence of the cationic lipid TDAB in gallic acid buffer solutions, the apparent pKa changes to 4.23. However, the pKa's are 4.31 in the presence of the cationic lipid trioctyl methyl ammonium chloride and 4.68 in the presence of the anionic lipid phosphoric acid di-n-tetradecyl ester. The membranes composed of the latter two lipids show very small electric responses to sucrose solution compared with those of tetradodecylammonium bromide,[4] indicating that the decrease in the pKa of gallic acid under the given conditions requires a change in electric potential with sugars.
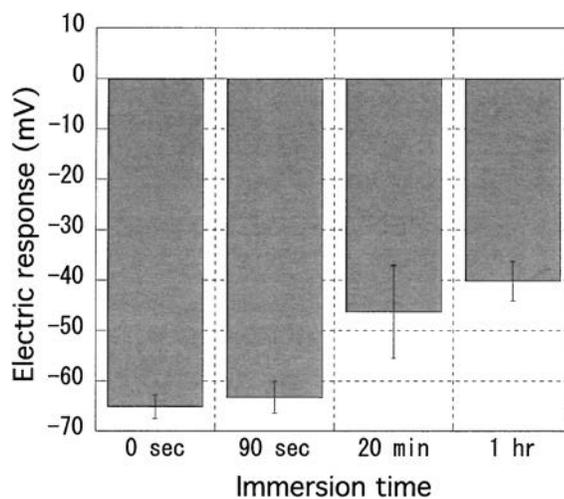
Fig. 6. Electric responses to 1 M sucrose solution. After preconditioning with gallic acid, the lipid/polymer membranes composed of 2.5 mg of TDAB, 1.5 ml of DOPP and 800 mg of PVC were immersed into the pH 12 solution (KOH) for 90 s, 20 min and 1 h, respectively.
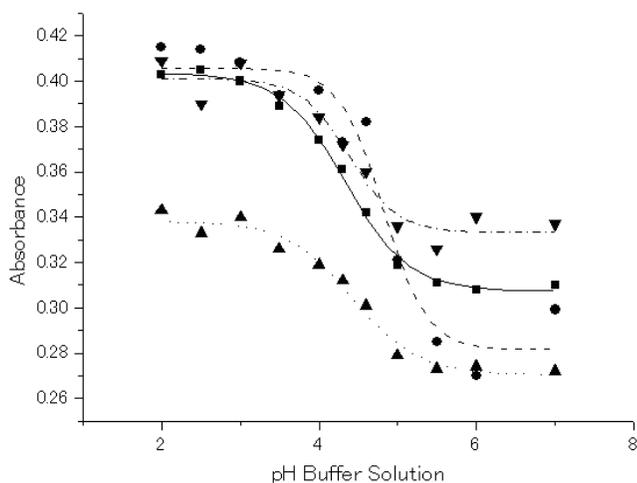

Fig. 7. Absorbance shift of 5×10^{-5} M gallic acid in buffer solution (inverted triangle), 3×10^{-4} M tetradodecylammonium bromide (square), 3×10^{-4} M trioctyl methyl ammonium chloride (triangle) and 3×10^{-4} M phosphoric acid di-n-tetradecyl ester (circle), scanned at different pHs.

3.   Conclusions

The lipid/polymer membrane modified with a phenolic compound has a high sensitivity to sugars. Our results obtained from UV spectra and the taste sensor indicate that the structural and chemical properties of amphiphilic compounds change the pKa of gallic acid. Further investigation of phenolic compounds and lipids is required to develop membranes with a high sensitivity to sugars.

Acknowledgement

References

1.   K. Toko: Biomimetic Sensor Technology (Cambridge University Press, Cambridge, 2000).
2.   M. Habara, H. Ikezaki, A. Taniguchi and K. Toko: IEEJ **121-E** (2001) 641 (in Japanese).
3.   M. Habara, H. Ikezaki and K. Toko: JASTS **7** (2001) 361 (in Japanese).
4.   M. Habara, H. Ikezaki and K. Toko: Biosens. Bioelectron. **19** (2004) 1559.
5.   A. E. Hagerman, K. M. Riedl and R. E. Rice: Basic Life Sciences **66** (1999) 495.
6.   K. Polewski, S. Kniat and D. Slawinska: Current Topics in Biophysics **26** (2002) 217.
7.   K. Hayashi, M. Yamanaka, K. Toko and K. Yamafuji: Sens. Actuators B **2** (1990) 205.
8.   K. Toko, K. Hayashi, M. Yamanaka and K. Yamafuji: Tech. Digest 9th Sens. Symp. (1990) 193.
9.   M. Watanabe, K. Toko, K. Sato, K. Kina, Y. Takahashi and S. Iiyama: Sensors and Materials **10** (1998) 103.
10.   M. Friedman and H. S. Jurgens: J. Agric. Food Chem. **48** (2000) 2101.
11.   IUPAC: Stability Constants of Metal-Ion Complexes, Part B, Organic Ligands (Pergamon Press, Oxford, 1979).