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Development and Investigation of a Sweetness Sensor for Sugars—Effect of Lipids—

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Several methods of taste evaluation, such as sensory tests and the use of electronic tongues and a taste-sensing system based on lipid/polymer membranes, have been developed and utilized in the food and pharmaceutical fields. In particular, the taste-sensing system can individually quantify five basic tastes using each type of sensor membrane. However, it is difficult to develop a sweetness sensor, because sweeteners cover a large number of compounds with diverse chemical structures and sizes. Using membrane potential measurements, the taste-sensing system needs three types of sensor membrane for each electric charge type (neutral, negative and positive) of sweetener. The sweetness sensor for uncharged sweeteners has been commercialized, but the mechanism of the response to sugars has not been clarified. Therefore, we investigated how the sensor responds to sugars in this study. As a result, we confirmed the unnecessity of the aromatic ring and that of the carboxyl group and the basic sensor-rinsing solution including cations, and concluded that both the hydrophobicity and electric charge of the surface of the sensor membrane influence the sweetness response.

1. Introduction

Sweetness indicates nutrient sources such as saccharides. Sweet substances cover a large number of compounds with diverse chemical structures and sizes, as represented by sugars (*e.g.*, sucrose), sugar alcohols (*e.g.*, mannitol), sulfonyl amides (*e.g.*, saccharine sodium), D-amino acids (*e.g.*, D-tryptophan), peptides (*e.g.*, aspartame) and proteins (*e.g.*, thaumatin). Sugars are one of the most famous groups of sweeteners. Only one type of heterodimeric receptor (T1R2+T1R3) responds to sweeteners with

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all chemical structures.[1,2] However, there are no significant characteristics common to only sweeteners. The AH-B theory is one of the most widely accepted models, but no model can explain the structural features common only among sweeteners without exceptions.[2–5] Hence, it is difficult to realize a sweetness sensor. Some methods of estimating sweetness have been developed and commercialized, for example, the Brix meter (refractometer) and NIR or FT-IR techniques.[6,7] These methods mainly estimate the quantities of sugars that are uncharged sweeteners, whereas they cannot measure all types of sweetener.

A taste-sensing system commercialized by Intelligent Sensor Technology, Inc. is one of the electronic tongues with global selectivity, which means that a taste sensor must respond consistently to only one basic taste (saltiness, sourness, umami, bitterness, or sweetness) despite the various chemical structures and sizes of tastants.[8–10] The taste-sensing system using a lipid/polymer membrane as a sensing part is a potentiometric measurement system using changes in the membrane potentials caused by tastants as sensor outputs. It is difficult to measure sweetness using only one sensor membrane because sweeteners have three types of electric charge (uncharged, positively charged and negatively charged). Therefore, three types of sweetness sensor have been developed for each type of electric charge.[11–14] The sweetness sensor for uncharged sweeteners (mainly sugars) has been commercialized, but the selectivity of this sensor to sweetness is not satisfactory at present.[11,12] To realize a sweetness sensor for uncharged sweeteners with high selectivity, we have investigated how the sensor responds to sugars. In previous studies,[11,12,15] the interactions between membrane components and sugars were investigated, and some hypotheses were suggested. One of them was that three factors, the carboxyl or phosphate group, the aromatic ring in sensor membranes and the basic sensor-rinsing solution including cations, were necessary for the sugar response. In this study, the effects of membrane components (mainly lipids with a carboxyl group and without an aromatic ring) on the responses of sensor membranes to sugars were investigated.

2. Experiment Methods

2.1 *Lipid/polymer membrane*

A lipid/polymer membrane comprising a lipid, a plasticizer and polyvinyl chloride (PVC) is used as both a sensing part and a transducer in the taste-sensing system. Lipid/polymer membranes respond to each basic taste depending on the concentrations and combination of the lipid and plasticizer. This characteristic is used to realize the taste sensor membrane with global selectivity.

Lipid/polymer membranes are electrically charged on their surface in aqueous solutions. In a solution containing electrolyte taste substances (salty, sour, and umami substances), electrolyte tastants electrically interact with an oppositely charged lipid/polymer membrane. These tastants are electrically attracted to the membrane surface and cause the change in membrane potential. In a solution containing electrically charged hydrophobic taste substances (*e.g.*, bitter and astringent substances), the tastants electrically and hydrophobically interact with an oppositely charged sensor membrane,

and thus they are attracted and adsorbed onto the membrane surface. The interaction between tastants and the sensor membrane is generally stronger than that without hydrophobic interaction. These hydrophobic substances often remain on the membrane surface after being simply washed by aqueous solutions. This characteristic is exploited to measure the change in membrane potential caused by the adsorption of tastants, which is called CPA, as detailed later.

The membrane potential of the taste sensor membrane is defined as the voltage between the sensor electrode and a reference electrode (Ag/AgCl electrode). The change in the membrane potential is calculated as the difference between the membrane potentials in a sample solution and a reference solution (30 mM KCl, 0.3 mM tartaric acid, aq).

2.2 *Measurement procedure*

Lipid/polymer membranes were fabricated by a conventional method.[8–14,16] PVC and adequate amounts of lipid and plasticizer were mixed for 1 h in 10 mL of tetrahydrofuran (THF, Sigma-Aldrich Co., LLC., St. Louis, MO, USA), depending on the taste sensor type. The mixture was desiccated in a Petri plate at room temperature for 72 h to form the transparent membrane.[16] The sensor membrane fabricated by this method can normally be used repeatedly (about 3000 times). Measurements were performed using the SA402B taste-sensing system (Intelligent Sensor Technology, Inc., Kanagawa, Japan). The measurement procedure was as follows. First, the membrane potential in the reference solution, V_r, was determined by potentiometry between sensor electrodes and a reference electrode. Next, the membrane potential in a sample solution, V_s, was measured. Then, the membrane potential in the reference solution was determined again (V_r'), after the sensor electrodes were washed with the reference solution (3 s × 2). Finally, the sensor electrodes were rinsed with a basic sensor-rinsing solution (30 vol% ethanol, 100 mM KCl, and 10 mM KOH). The difference between V_s and V_r, that is, $V_s - V_r$, is defined as a relative value. The difference between V_r' and V_r, that is, $V_r' - V_r$, is defined as a CPA value (CPA: change in membrane potential caused by adsorption).[8,9] This procedure was repeated twenty times for each sample, and the relative values in the twentieth cycle were used as the relative values of each sample. Four sensor probes were used for each sensor membrane type ($n = 4$). The averages and standard deviations of the four sensor outputs were used as the values and error bars in each figure.

2.3 *Selection of plasticizers*

Lipid/polymer membranes comprising palmitic acid (PA, Wako Pure Chemical Industries, Ltd., Osaka, Japan) as a lipid, PVC and one of the three plasticizers were fabricated. PA is a fatty acid and has negative charges in an aqueous solution. The three plasticizers used in this selection were as follows: tributyl O-acetyl citrate (TBAC, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), bis(1-butylpentyl) adipate (BBPA, Sigma-Aldrich Co., LLC., St. Louis, MO, USA) and phosphoric acid tris(2-ethylhexyl) ester (PTEH, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). The quantities of these plasticizers were the same for each membrane (1.0 ml). All the lipids and plasticizers adopted in the experiment included no aromatic ring in their chemical structures. These

twelve types of lipid/polymer membrane (four amounts of PA × three types of plasticizer) were used as the sensor membranes of four sensor electrodes to measure four sucrose samples with different concentrations (100, 300, 500, and 1000 mM).

2.4 *Effect of lipid quantities on relative values*

Lipid/polymer membranes comprising PA and tetradodecylammonium bromide (TDAB, Sigma-Aldrich Co., LLC., St. Louis, MO, USA) as lipids, PVC and a plasticizer, chosen on the basis of the results described in § 2.3, were fabricated. There were nine different quantities (0.01–1.0 mg) of TDAB included in the sensor membranes, which also contained PA (0–30 mg). Four sucrose samples (100, 300, 500, and 1000 mM) were measured as target samples. Relative values were used as sensor outputs.

3. Results and Discussion

3.1 *Selection of plasticizers*

To select the most suitable plasticizer for the sweetness sensor, we investigated three types of plasticizer, PA as a lipid and PVC as a polymer. Using each plasticizer with PA in each membrane, we fabricated twelve membranes, as shown in Table 1, and measured the four sucrose samples using them. The results of the measurements are shown in Figs. 1(a)−1(d). In the measurement using the sensor membranes with no PA, there was no response to the sucrose samples. In the measurement using the sensor membranes containing 10−30 mg of PA, on the other hand, the responses to the sucrose samples clearly indicated sucrose concentration dependence; the relative value increased with increasing sucrose concentration. In particular, in the measurement using the sensor membranes containing 20−30 mg of PA, the sensor membranes that include TBAC as a plasticizer showed a higher response to the sucrose samples than those that include PTEH or BBPA. Therefore, TBAC was considered as the best plasticizer for the sweetness sensor membrane among the three plasticizers investigated.

Additionally, the effect of the carboxyl group in the chemical structures of membrane components was investigated. Figure 2 shows the responses of three types of sensor membrane to sucrose. One of them was fabricated using unpurified BBPA as a plasticizer, another one was fabricated using purified BBPA as a plasticizer, and the third one was fabricated using purified BBPA with adipic acid. Adipic acid, which has two carboxyl groups in its chemical structure, can be speculated as a main impurity of

Table 1
Twelve membranes containing PA.

No TDAB	Plasticizer (1.0 ml)		
PA (mg)	TBAC	BBPA	PTEH
0		Fig. 1(a)	
10		Fig. 1(b)	
20		Fig. 1(c)	
30		Fig. 1(d)	

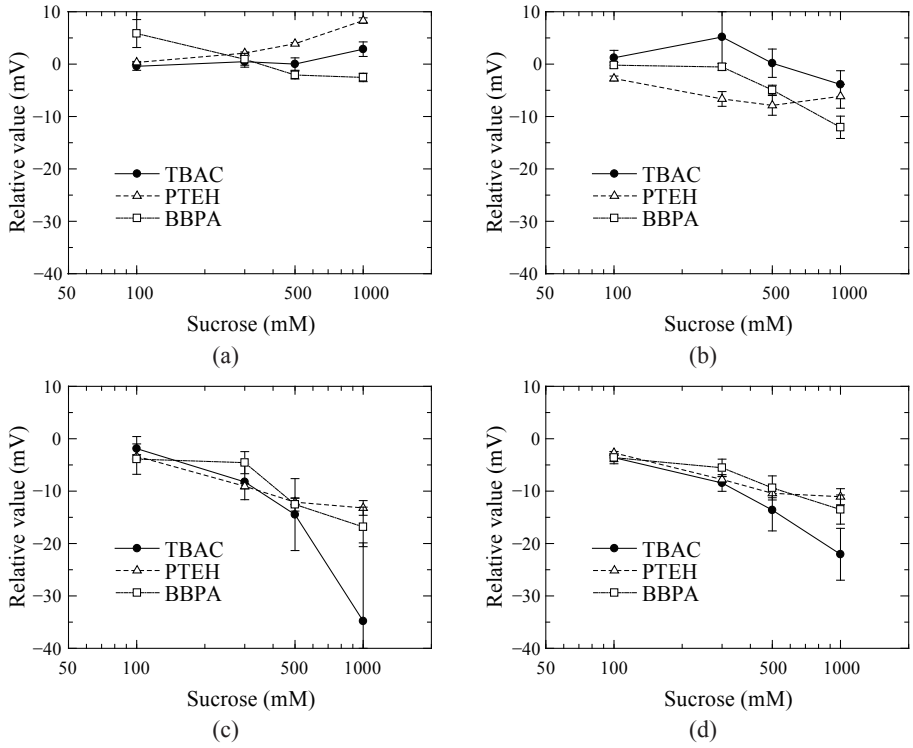

Fig. 1. Sweetness responses of the membranes containing PA. (a) No PA, (b) 10 mg of PA, (c) 20 mg of PA, and (d) 30 mg of PA.

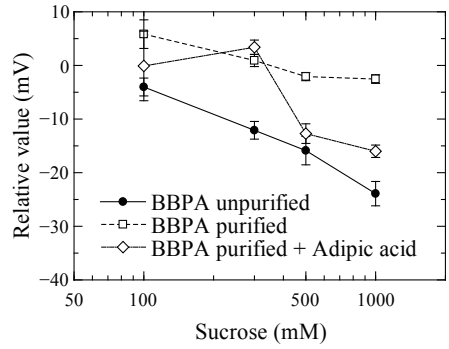

Fig. 2. Sweetness responses of the membranes using BBPA.

BBPA considering its production method. Only the membrane using purified BBPA indicated no response to sucrose, as shown in Fig. 2. Simultaneously, we also measured the sucrose samples using the same three types of sensor membrane and washed them in a neutral sensor-rinsing solution, which does not include any cation, instead of the

commonly used basic sensor-rinsing solution including cations. As a result, all sensor membranes showed no responses to sucrose. Figure 3 shows the effects of the sensor-rinsing solutions on the sweetness responses measured by the membranes using purified BBPA and adipic acid. These results indicated that aromatic rings in the chemical structures of membrane components were not necessary and that both the carboxyl group and the basic sensor-rinsing solution including cations were necessary for sugar responses. The hypothesis in previous studies was partially negated and partially affirmed.

3.2 *Effects of lipid quantities on relative values*

Next, we investigated the effects of the quantities of two lipids (PA and TDAB) on the sucrose response. The results of the measurements, as shown in Table 2, are shown in Figs. 4−6. From the conclusion in Fig. 1, all the membranes included TBAC as a plasticizer. The membranes containing 20 mg of PA showed responses to sucrose samples depending on the TDAB concentration, as shown in Fig. 4. The responses have a peak at approximately 0.2 mg of TDAB (about −50 mV to 1000 mM sucrose). This is the largest response to 1000 mM sucrose among the sensor membranes investigated in this study. Figure 5 indicates the response of the membrane containing 0.2 mg of TDAB to 1000 mM sucrose, which is the largest, as shown in Fig. 4. The response of the membrane containing 20 of mg PA is higher than that of the membrane containing 10 or

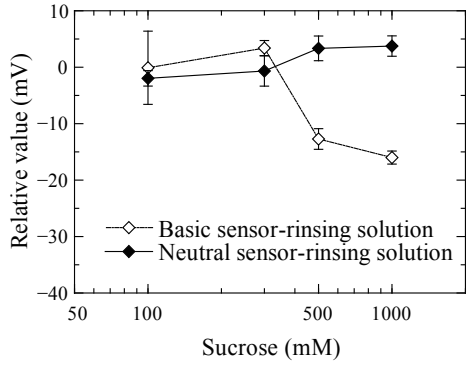

Fig. 3. Effects of the sensor-rinsing solutions on the sweetness responses.

Table 2
Membranes containing PA, TDAB, and TBAC.

TBAC		TDAB (mg)								
		0.01	0.02	0.03	0.05	0.1	0.2	0.3	0.5	1
PA (mg)	10						Fig. 6.			
	20					Fig. 5.				
	30		Fig. 4.							

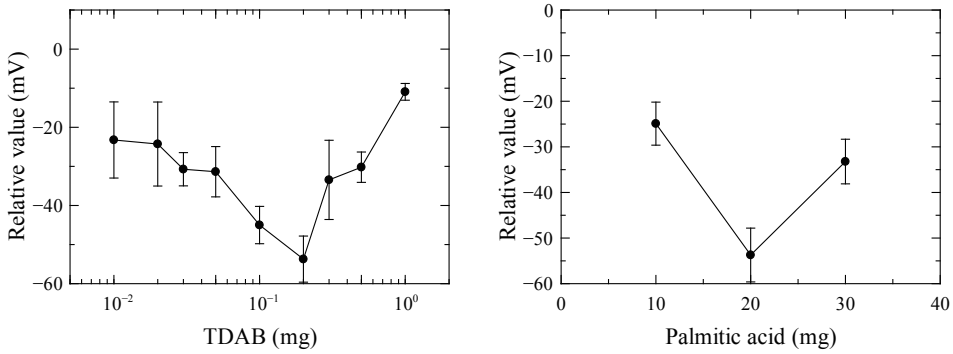

Fig. 4 (left). Sweetness responses of the membranes containing TDAB and 20 mg PA.
Fig. 5 (right). Sweetness responses of the membranes containing 0.2 mg TDAB and PA.

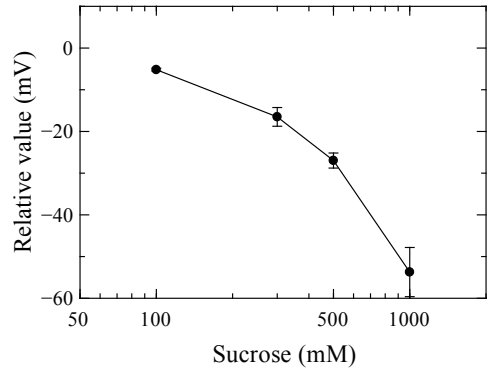

Fig. 6. Concentration dependence of sweetness responses of the membranes containing 0.2 mg of TDAB and 20 mg of PA.

30 mg of PA. From these results, we studied the membrane comprising 0.2 mg of TDAB and 20 mg of PA, and found that the response increased with the sucrose concentration, as shown in Fig. 6.

Generally, a peak shown in the lipid quantity dependence of the sensor response is caused by two factors, i.e., hydrophobicity and electric charge, as discussed in previous papers.[17–19] When the lipid quantity increases, the increasing electric charge of the membrane surface causes an increase in the adsorption of taste substances by electrical interaction. On the other hand, decreasing the hydrophobicity of the membrane surface causes a change in the intensity of the hydrophobic interaction between the sensor membrane and the taste substance. The peak that appeared in Figs. 4 and 5 can be considered as a result of the effects of both the hydrophobicity and electric charge of the surface of the sensor membrane. The quantity of TDAB exerted a large effect on both the electric charge and hydrophobicity of the membrane, because it was one of the quaternary ammonium salts that completely ionized in aqueous solutions. The quantity of PA mainly exerted an effect on the hydrophobicity of the membrane, because it has a carboxyl group that does not completely ionize in aqueous solutions.

4. Conclusions

A sweetness sensor for sugars was investigated in this study. The selection of plasticizers was carried out and the effects of lipid quantities were investigated. As a result, we partially negated (necessity of aromatic rings) and partially affirmed (necessity of the carboxyl group and the basic sensor-rinsing solution including cations) our previous hypothesis, which was presented in our previous studies. We conclude that both the hydrophobicity and electric charge of the surface of the sensor membrane influence the sweetness response. In addition, we developed a sweetness sensor that showed an over −50 mV response to 1000 mM sucrose and sucrose-concentration-dependent responses. One of our future tasks is to clarify the role of the carboxyl group in the mechanism of sweetness response.

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