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Sweetness Sensor with Lipid/Polymer Membranes: Sweet-Responsive Substances

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A sweetness sensor with lipid/polymer membranes has been developed for evaluating the sweetness of sugars and sugar alcohols. Among the constituents of lipid/polymer membranes, gallic acid has been used as the main substance involved in sucrose response in our group. In this study, as a step toward understanding the response mechanism of the sweetness sensor, functional groups of gallic acid, namely, carboxyl and hydroxyl groups, were focused on. The results demonstrated that the carboxyl group is essential for the sweetness sensor, whereas the hydroxyl group is not always necessary for the sucrose response. It was also revealed that the phosphate group may be a substitute for the carboxyl group. Then, for one of the sensors with the highest response to a 300 mM sucrose solution, named the sweetness sensor GL1, the basic characteristics such as selectivity and correlation with sweetness were investigated. The behavior of GL1 sensor outputs was relatively similar to the sweetness perception in humans.

1. Introduction

It has been difficult to develop sweetness sensors with a lipid/polymer membrane because sweet-tasting substances like sucrose do not have an electric charge. To date, by changing the compositions of sensors with a lipid/polymer membrane, the selectivity and sensitivity to a sucrose solution have been gradually improved by our group.[1–3] One of the recent results was that the contact of sucrose to the surface of a lipid/polymer membrane depends on the chemical structure of the surface-modifying phenolic compound like gallic acid.[4] For all the data obtained in these studies, the surface-modified lipid/polymer membranes were used.

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Gallic acid, which is an important constituent of the sweetness sensor, has two functional groups, i.e., carboxyl and hydroxyl groups. In this paper, from the viewpoint that functional groups play important role(s) in the interaction between the sweetness sensor and sugar samples, substances that are similar to gallic acid and have various functional group(s) were checked for their sucrose response. The substances capable of producing a sucrose response in the sweetness sensor were tentatively named SRS, for "sweet-responsive substance."

It is important to determine the necessary and sufficient conditions for the sweetness sensor, which may provide hints for understanding the response mechanism. This may be a basis for creating sensors that can detect neutral substances other than sugars.

2. Materials and Methods

2.1 *Sensor with lipid/polymer membrane*

Sensors with lipid/polymer membranes were made using tetradodecylammonium bromide (TDAB), 1.5 mL of di-n-octylphenylphosphonate (DOPP), and 800 mg of polyvinyl chloride (PVC) as lipid, plasticizer, and polymer, respectively, as described previously.[1,3] Basically, the amount of TDAB added in lipid/polymer membranes was 1 mg, by which the maximum electric response was obtained. However, some lipid/polymer membranes showed a maximum electric response with 0.5 to 2 mg of TDAB; 0.5 mg of TDAB was added for lipid/polymer membranes with 4-hexadecylsulfonyl-3-nitrobenzoic acid, 1.2 mg of TDAB for those with 3,4-didodecyloxybenzoic acid (DDoBA), and 1.25 mg of TDAB for those with polymer with carboxylic acid 1. Moreover, 1.5 mg of TDAB was added for those with 4-(hexadecylsulfonyl)benzoic acid, polymer with carboxylic acid 3, and phenylphosphonic acid, and 2 mg of TDAB was added for those with gallic acid, 4-methoxyphenol, 4-methoxybenzoic acid, phthalic acid, 4-methoxyphthalic acid, and 1-naphtylphosphoric acid.

2.2 *Sweet-responsive substances (SRSs)*

For sweet-responsive substances (SRSs), trimellitic acid, gallic acid, 3,4-dihydroxybenzoic acid, caffeic acid, 1,2,3-benzenetricarboxylic acid, 4-hydroxyisophthalic acid, phthalic acid, isophthalic acid, terephthalic acid, 3,4,5-trimethoxybenzoic acid, 4-methoxyphthalic acid, oleic acid, phenylphosphonic acid, and 1-naphtylphosphoric acid were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Methyl gallate, 4-methoxyphenol, 4-methoxybenzoic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid, 1,4-naphthalenedicarboxylic acid, 4-aminobenzoic acid, 4-amino-3-hydroxybenzoic acid, 3-chloro-4-hydroxybenzoic acid, 4-(hydroxymethyl) benzoic acid, 3,4-dimethoxycinnamic acid, 3,4,5-trimethoxycinnamic acid, 4-hydroxyphenylacetic acid, 4-(hexadecylsulfonyl) benzoic acid, 4-hexadecylsulfonyl-3-nitrobenzoic acid, polymer with carboxylic acid 1 (poly(methyl methacrylate-co-methacrylic acid)), polymer with carboxylic acid 2 (poly(ethylene-co-acrylic acid)), and polymer with carboxylic acid 3 (poly[2,6-bis(hydroxymethyl)-4-methylphenol-co-4-hydroxybenzoic acid]) were purchased from Sigma-Aldrich, USA. Palmitic acid was purchased from Wako Pure Chemical Industries, Ltd., Japan. DDoBA was synthesized from 3',4'-(didodecyloxy)benzaldehyde,

which was purchased from Epsilon Chimie, France, by oxidation with sodium chlorite and 2-methyl-2-butene in tert-BuOH/H2O/THF (5:1:4).

The amounts of SRS added in lipid/polymer membranes are described in Table 1. As for the polymer with carboxylic acids 1–3 in Table 1, the amount of PVC added in a lipid/polymer membrane was decreased so that the total amount of polymer became 800 mg, because they are both polymer and SRS.

Table 1
Electric response to sucrose solution.

No.	Sweet-Responsive Substance (SRS)		Electric response (mV)	
			300 mM Sucrose	1 M Sucrose
1	Trimellitic acid	100 mg	−40.0	−64.0
2	Gallic acid	100 mg	−23.0	−63.0
3	3,4-Dihydroxybenzoic acid	100 mg	−36.5	−59.2
4	Caffeic acid	100 mg	−40.0	−63.0
5	3,4-Dihydroxyphenylacetic acid	100 mg	−31.0	−53.0
6	4-Hydroxybenzoic acid	100 mg	−23.3	−59.0
7	1,2,3-Bezenetricarboxylic acid	100 mg	−19.1	−51.5
8	1,4-Naphthalenedicarboxylic acid	100 mg	−28.4	−66.3
9	4-Hydroxyisophthalic acid	30 mg	−30.0	−63.6
10	Phthalic acid	30 mg	−19.2	−79.7
11	Isophthalic acid	100 mg	−14.2	−40.3
12	Terephthalic acid	100 mg	−23.1	−43.7
13	3,4,5-Trimethoxybenzoic acid	150 mg	−21.7	−62.9
14	4-Aminobenzoic acid	100 mg	−21.0	−52.4
15	4-Amino-3-hydroxybenzoic acid	100 mg	−20.0	−41.8
16	3-Chloro-4-hydroxybenzoic acid	30 mg	−29.2	−70.5
17	4-(Hydroxymethyl)benzoic acid	100 mg	−24.8	−59.2
18	3,4-Dimethoxycinnamic acid	100 mg	−22.5	−53.8
19	3,4,5-Trimethoxycinnamic acid	100 mg	−20.8	−61.1
20	4-Hydroxyphenylacetic acid	100 mg	−29.3	−60.7
21	4-Methoxyphthalic acid	100 mg	−27.7	−67.9
22	4-(Hexadecylsulfonyl)benzoic acid	3 mg	−15.7	−49.9
23	4-Hexadecylsulfonyl-3-nitrobenzoic acid	1 mg	−14.8	−37.2
24	3,4-Didodecyloxybenzoic acid (DDoBA)	10 mg	−10.3	−50.4
25	Polymer with carboxylic acid 1	8 mg	−6.4	−34.3
26	Polymer with carboxylic acid 2	8 mg	−13.5	−43.7
27	Polymer with carboxylic acid 3	0.8 mg	−13.0	−50.2
28	Palmitic acid	10 mg	−19.4	−48.5
29	Oleic acid	10 mg	−20.7	−50.2
30	Phenylphosphonic acid	150 mg	−13.4	−39.2
31	1-Naphtylphosphoric acid	30 mg	−5.9	−30.9

2.3 *Measurement conditions*

Sensors with lipid/polymer membranes were preconditioned with the reference solution (30 mM KCl and 0.3 mM tartaric acid, pH 3.5) for 48 h before use. Measurements were performed using the Taste Sensing System SA402B (Intelligent Sensor Technology, Inc., Japan), as described previously.[5] For cleaning the sensors, a positively charged membrane-washing solution (10 mM NaOH, 100 mM NaCl, 30% EtOH, pH 12.5) was used. This solution is important not only for washing but also for optimizing the sensor. Because the surfaces of sensors always have a positive electric charge, data are obtained as negative values; the higher the concentration of the sucrose solution, the more negative the sensor output value. Data are shown as average values from the last three out of five cycles of measurement, except for DDoBA (the last three out of ten cycles of measurement), because sensor outputs from the early stage of measurement tend to be unstable.

2.4 *Measurement samples*

Sample solutions of sugars were prepared by adding them to the reference solution (30 mM KCl and 0.3 mM tartaric acid, pH 3.5). Sucrose, glucose, fructose, xylitol, erythritol, sorbitol, mannitol, maltose, lactose, trehalose, palatinit, raffinose, isomalt-oligosaccharide, and fructo-oligosaccharide were purchased from Wako Pure Chemical Industries, Ltd., Japan. For sample solutions of basic tastes, potassium chloride (300 mM), tartaric acid (3 mM), monosodium glutamate (10 mM), and quinine hydrochloride (0.1 mM) were used as salty, sour, savoriness (umami), and bitter samples, respectively.

3. Results and Discussion

3.1 *Electric response of sensors with gallic acid and its derivatives*

In contrast to the previous studies,[1–4] a new method was used in this paper, in which no surface modification of the sensor is performed before measurement. This is because of the preliminary results, suggesting that surface-modified lipid/polymer membranes and the membranes proposed here often have different sucrose responses. Since some phenolic compounds showed a sucrose response only when they were contained within the lipid/polymer membranes, one of the reasons for the difference was considered to be the difference in the efficiency of surface modification among the phenolic compounds.

As a functional group, the carboxyl and hydroxyl groups from the chemical structure of gallic acid were focused on. The results are summarized in Fig. 1. Firstly, to evaluate the importance of the carboxyl group, methyl gallate was used as an alternative to gallic acid, which has a methylester moiety instead of a carboxyl group. The electric response of the sensor with methyl gallate was considerably reduced compared with that with gallic acid (Fig. 1).

Secondly, to evaluate the importance of the hydroxyl group, 3,4,5-trimethoxybenzoic acid was used as an alternative to gallic acid, which has a methoxy group instead of a hydroxyl group. The electric response of the sensor with 3,4,5-trimethoxybenzoic acid was almost the same as that with gallic acid (Fig. 1). To evaluate again the importance of the carboxyl and hydroxyl groups, 4-hydroxybenzoic acid (and its derivatives) was also used as an alternative to gallic acid. The results indicated that the derivative without

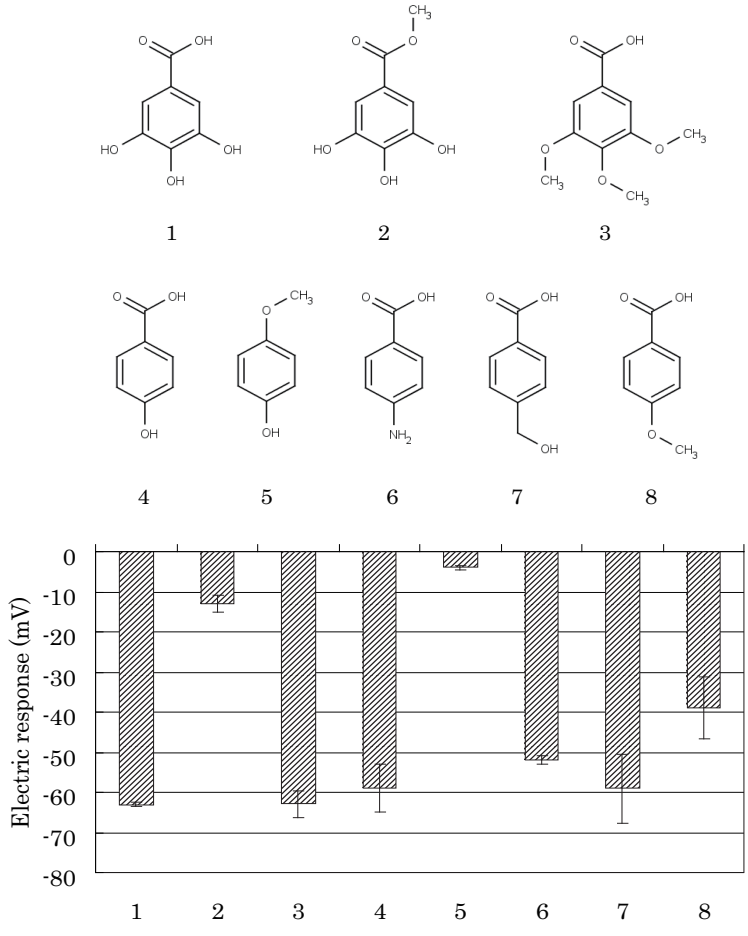

Fig. 1. Electric responses to 1 M sucrose of sensors with substances analogous to gallic acid. (1) gallic acid, (2) methyl gallate, (3) 3,4,5-trimethoxybenzoic acid, (4) 4-hydroxybenzoic acid, (5) 4-methoxyphenol, (6) 4-aminobenzoic acid, (7) 4-(hydroxymethyl)benzoic acid, (8) 4-methoxybenzoic acid.

the carboxyl group does not have any sucrose response, whereas those without the hydroxyl group showed a sucrose response relatively equal to that of 4-hydroxybenzoic acid. These results suggest that the carboxyl group is essential for the sucrose response, whereas the hydroxyl group is not.

3.2 *Electric response of various substances having carboxyl group*

Electric responses were further checked using various substances with carboxyl group(s) similar to gallic acid (Table 1). Included were only those producing a certain level of sucrose response. Because of the previous results[4] and preliminary results

showing that almost all the substances with a carboxyl group neighboring hydroxyl group(s) on the benzene ring, such as 2,6-dihydroxybenzoic acid, seldom give a sucrose response, they were not included as test substances. For each substance tested, several (or more) sensors were made so that the maximum response was obtained; in some cases, a 30 mg amount gave the highest response.

The results clearly showed that the sucrose response is observed not only in substances with both carboxyl group and hydroxyl group on the benzene ring but also in those with only the carboxyl group such as 1,4-naphthalenedicarboxylic acid and phthalic acid. It was also indicated that the phosphate group is also capable of producing the sucrose response, as well as the carboxyl group. Among the substances tested, on the other hand, those with the sulfo group on the benzene ring gave a low sucrose response (data not shown), which may suggest that a weak acid is important for the sucrose response. Although lower than those of others, the sucrose response was also observed for palmitic acid and oleic acid, which suggests that the benzene ring is not always necessary. Interestingly, the results also demonstrated that even the sensors with carboxyl groups in their polymer portion show the sucrose response, although the level is not high.

All these results suggest that the carboxyl group or phosphate group is essential for the sucrose response and that whatever substance with such a functional group may function as SRS. Considering these results, to indicate substances producing the sucrose response such as gallic acid, the term "phenolic compound," which has been used before,[3,4] seems no longer applicable. Thus, the substances capable of producing the sucrose response for the sweetness sensor were tentatively named SRS, for "sweet-responsive substance."

Because it became clear that trimellitic acid is one of the best SRSs with regard to electric response to a dilute solution of 300 mM sucrose, it was exclusively used for the following experiments. Another reason is that the chemical structure of trimellitic acid is simple; it is composed of a benzene ring only with three carboxyl groups. To simplify, the sensor with trimellitic acid is hereafter named sweetness sensor GL1.

3.3 *Basic characteristics of GL1 sensor*

The sucrose response of the GL1 sensor was examined with regard to pH (Fig. 2). The results showed that the level of electric response was decreased at pH 4 or lower but increased at pH 10 or higher. This behavior looks similar to the sweetness perception in humans, as far as the pH of common foods, namely, around pH 2 to pH 7, is concerned. This is, in other words, concerned with the interaction between sweetness and sourness. In general, we are supposed to perceive sweet more weakly with the addition of sourness.

Next, the concentration characteristics of the GL1 sensor were analyzed using four sugars (Fig. 3). For all the sugar samples, electric responses start with approximately 10 –30 mM and increase logarithmically with an increase in concentration to approximately 100–300 mM. Then, they decrease at approximately 1,000 mM or higher. This behavior of the GL1 sensor is quite similar to that of human perception; the human threshold for perceiving the sweet taste of a sucrose solution has been reported to be 10–30 mM,[6] and the concentration at which we can perceive most clearly is around 300 mM (ca. 10% w/w).[7] For a 1,000 mM sucrose solution, most people feel its sweetness too strongly to distinguish.

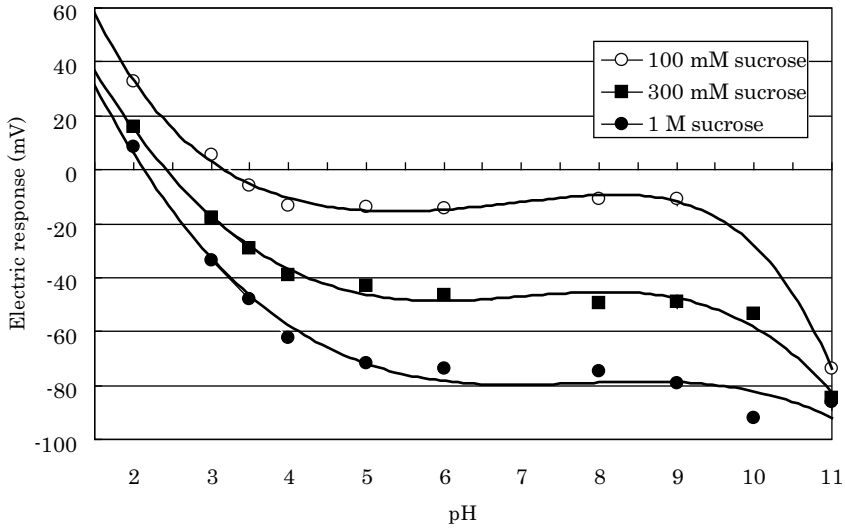

Fig. 2. Electric response of GL1 sensor to sucrose solution with various pHs.

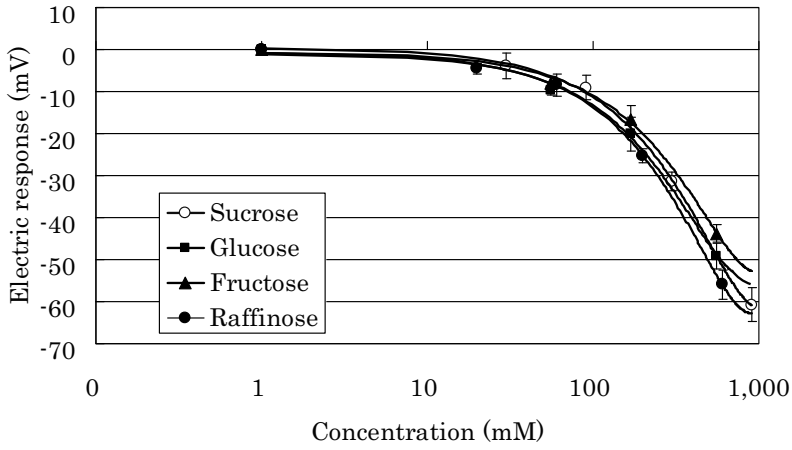

Fig. 3. Electric responses of GL1 sensor to four sugars.

Then, the sensitivity and selectivity of the GL1 sensor were checked using solutions of basic tastes, in comparison with the prototype sweetness sensor GL0 (Fig. 4). For 100 or 300 mM sucrose solutions, the sensitivity of the GL1 sensor is better than that of the GL0 sensor, although they were almost the same for the 1,000 mM solution. This clearly

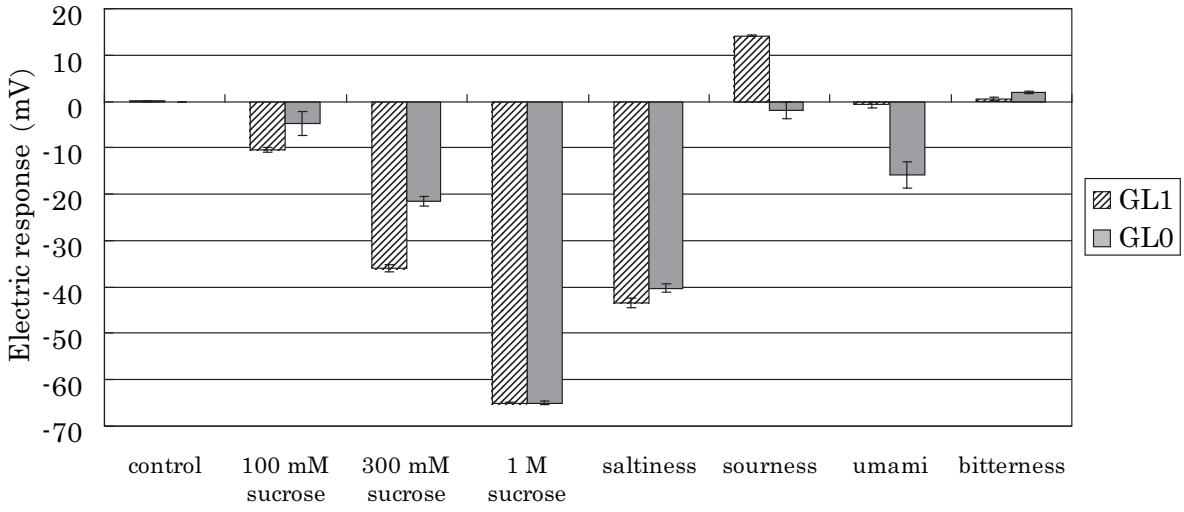

Fig. 4. Electric response of GL1 sensor to basic tastes.

indicates that the GL1 sensor is more effective, particularly for dilute solutions, than the GL0 sensor. Figure 4 also demonstrates that the selectivity of the GL1 sensor is better than that of the GL0 sensor, as long as the same materials are used. At least, it seems safe to say that the GL1 sensor is better than the GL0 sensor because it is not sensitive to umami solution (monosodium glutamate in this study), which is present in many foodstuffs; we often cannot distinguish taste qualities between sweetness and umami in some cases.[8] As for saltiness, the electric response for saltiness was unavoidable for the GL1 sensor, similarly to the GL0 sensor. However, the behavior of the GL1 sensor under the influence of sodium chloride sometimes fits with the human sense, both for dilute and concentrated solutions of sodium chloride (data not shown). Therefore, it seems that the effect of salts is not always a drawback.

Finally, as shown in Fig. 5, the electric responses of various sugars were measured using the GL1 sensor at 10% concentration (w/w), in comparison with their relative sweetness, which is based on human sensory evaluation.[7] The reason why the concentration in w/w was used only in this experiment, unlike in Figs. 1–4, is to compare with their relative sweetness. The results suggested that the highest levels of electric responses are observed for monosaccharides such as glucose and fructose, the second highest are for disaccharides such as maltose and trehalose, and the lowest are for oligosaccharides. This is similar to human sense, that is, in terms of relative sweetness. The correlation coefficient between the electric response of the GL1 sensor and the relative sweetness was 0.76, which is somewhat high.

However, conflicting results were obtained from the one-to-one comparison of sugars, such as the comparison between glucose and sucrose. One of the reasons is

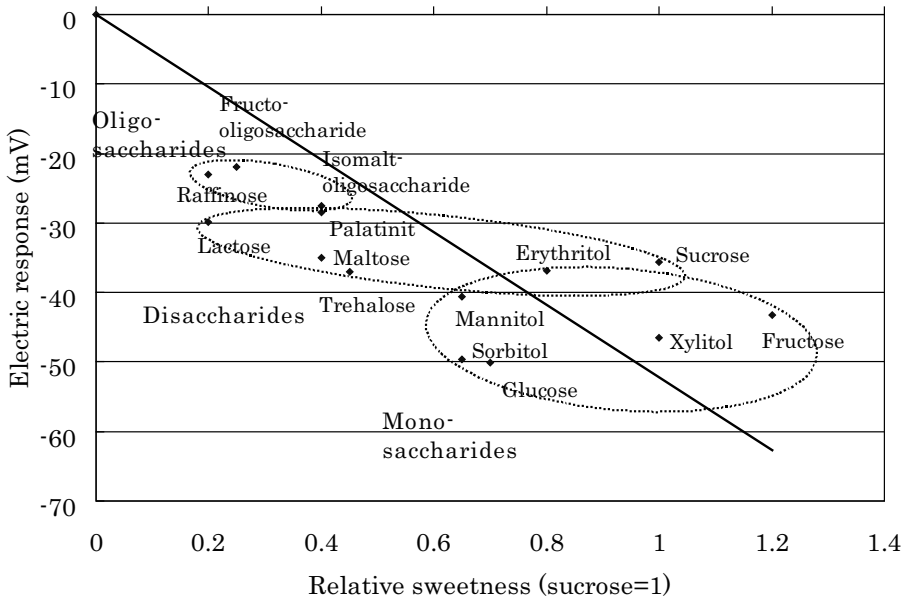

Fig. 5. Electric response of GL1 sensor to various sugars and sugar alcohols, in comparison with their relative sweetness.

that the relative sweetness of sucrose is exceptionally higher than those of the other disaccharides, the relative sweetness of which is 0.2–0.4. Still, Fig. 5 suggests that the GL1 sensor has a feature of "global selectivity," which is needed for the taste sensor,[5] as far as the sweetness of sugars and sugar alcohols is concerned.

4. Conclusions

It was suggested that the carboxyl or phosphate group in lipid/polymer membranes is essential for the sucrose response of the sweetness sensor, while the hydroxyl group is not always necessary. It was revealed that trimellitic acid is one of the best SRSs. The characteristics of this sensor incorporating trimellitic acid are relatively similar to those of human sense.

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