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Sweetness Sensor with Lipid/Polymer Membranes: Response to Various Sugars

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A sweetness sensor with lipid/polymer membranes has been developed for evaluating the sweetness of sugars and sugar alcohols. In this paper, experiments were performed to compare the electric responses of the sweetness sensor with the chemical structure of various sugars. The results demonstrated that the presence of two adjacent hydroxyl groups in a sugar molecule is important and that the optimum distance between the two adjacent hydroxyl groups is approximately 3 Å. The interaction between the sweetness sensor and sugars is discussed.

1. Introduction

It was difficult to develop a sweetness sensor with a lipid/polymer membrane because sweet-tasting sugars are nonelectrolytes. Although the mechanism underlying the response to sucrose is yet unknown, the sensitivity and selectivity of the sweetness sensor have been gradually improved.[1–5] The purpose of this study is to move toward understanding the response mechanism.

For sweetness perception in humans, the AH-B theory is one of the best-accepted models, which explains the relationship between sweet-tasting molecules and sweetness

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on the basis of hydrogen bonding.[6–8] The sweetness of most sweet-tasting molecules is applicable to the AH-B model, although some exceptions exist such as β-mannose, which is not sweet but bitter. Then, the most recent and complicated model was proposed by Nofre and Tinti, which assumes 6 additional recognition sites with the AH-B model so that the sweetness of almost all sweet-tasting molecules can be explained.[9] Such studies on structure-activity relationships (SAR) have been widely carried out until, finally, the sweet taste receptor was identified in the early 2,000s.[10] It is becoming clear that only a single sweet taste receptor responds to almost all sweet-tasting substances, including sweet proteins, sweet amino acids, and high-potency sweeteners, as well as sugars.[10] As far as sugars as sweet-tasting molecules are concerned, the only evidence of sweet taste receptor binding sites was reported by Nie *et al*., demonstrating that sucrose and glucose bind to the N-terminal domain of its two subunits (T1R2 and T1R3) with different affinities; sucrose binds more readily to the T1R3 subunit whereas glucose binds more readily to the T1R2 subunit.[11]

In any case, sweet-tasting sugars have hydroxyl groups, which appear to play important role(s) in the interaction with the human receptor. The purpose of this study is to investigate whether this is also the case with the sweetness sensor that has been developed recently.[5,12]

2. Materials and Methods

2.1 *Measurement using sweetness sensor*

The sweetness sensor GL1, which consists of trimellitic acid as a sweet-responsive substance (hereafter referred to as "SRS"), was made in the same manner as described in the same issue of this journal.[12] Before measurement, the sensor was preconditioned with the reference solution (30 mM KCl and 0.3 mM tartaric acid, pH 3.5) for 48 h. Measurements were performed using the Taste Sensing System SA402B (Intelligent Sensor Technology, Inc.), as described previously.[5,12] Since the surface of the sensors always has 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, because sensor outputs from the early stage of measurement tend to be unstable.

2.2 *Measurement samples*

Sample solutions of various sugars or their derivatives 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, maltose, lactose, trehalose, and raffinose were purchased from Wako Pure Chemical Industries, Ltd., Japan. Ethylene glycol, glycerol, 2-deoxyglucose, xylose, galactose, 2-deoxygalactose, mannose, lyxose, *trans*-1,2-cyclohexanediol, *cis*-1,2-cyclohexanediol, 1,3-cyclohexanediol (mixture of *cis* and *trans*), and 1,4-cyclohexanediol (mixture of *cis* and *trans*) were purchased from Tokyo Chemical Industry Co., Ltd., Japan. *cis*-1,2-Cyclopentanediol and *trans*-1,2-cyclopentanediol were purchased from Sigma-Aldrich, USA.

3. Results and Discussion

3.1 *Electric response of sweetness sensor GL1 to various sugars*

To eventually clarify the response mechanism of the sweetness sensor, particularly from the viewpoint of chemical structures, sugar samples were focused on in this study. First, seven major sugars were measured at 300 mM concentration (Fig. 1). The results indicated that, roughly, the bigger the sugar molecule, the bigger the electric response. At least, the electric responses of monosaccharides such as fructose and glucose were clearly lower than those of disaccharides such as maltose and sucrose. As long as the

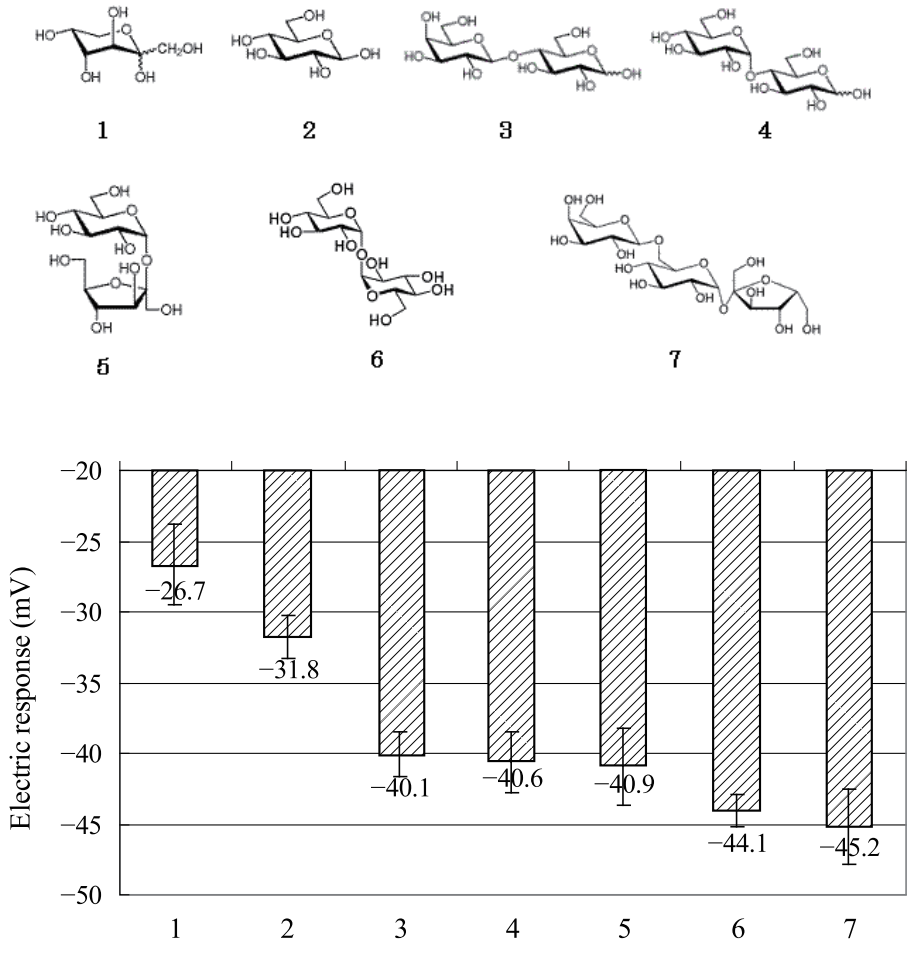
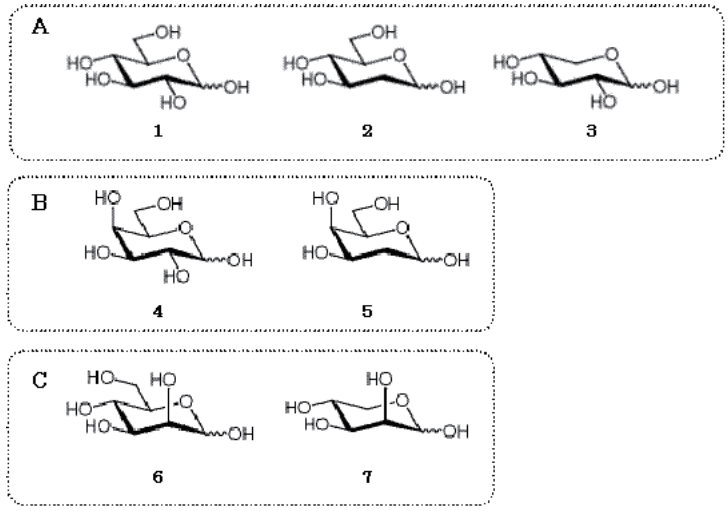


Fig. 1. Electric response of sweetness sensor GL1 to 300 mM sample solution; (1) fructose, (2) glucose, (3) lactose, (4) maltose, (5) sucrose, (6) trehalose, (7) raffinose.

mole concentrations are the same among the sugars, the bigger the molecule, the more the number of hydroxyl groups. Therefore, it was suggested that the more the number of hydroxyl groups, the higher the electric response.

Secondly, to examine the effect of the hydroxyl group, sugars with fewer hydroxyl groups were used as samples. Considering that a simple comparison among all the sugars is impossible because of the effect of anomeric conversion, the sugars were grouped into three, A to C (Fig. 2). As for group A, the chemical structures of 2-deoxyglucose



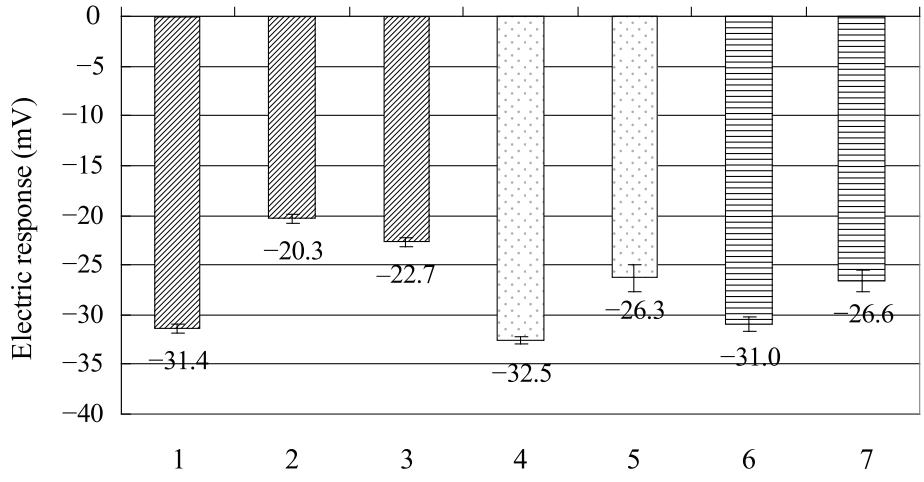


Fig. 2. Electric response of sweetness sensor GL1 to 300 mM sample solution; (1) glucose, (2) 2-deoxyglucose, (3) xylose, (4) galactose, (5) 2-deoxygalactose, (6) mannose, (7) lyxose.

and xylose are completely the same as that of glucose, except for the number of hydroxyl groups. Similarly, as for group B, the chemical structure of 2-deoxygalactose is completely the same as that of galactose, except for the number of hydroxyl groups, and as for group C, the chemical structure of lyxose is completely the same as that of mannose, except for the number of hydroxyl groups. The results demonstrated that, for all the patterns of comparison, a decrease in electric responses was observed for sugars with fewer hydroxyl groups.

Thirdly, to examine further the effect of the number of hydroxyl groups, substances with hydroxyl groups fewer than those of monosaccharides were used for the measurement using the GL1 sensor. Since they are linear molecules, linear sugars such as xylitol and sorbitol were chosen for comparison. The results indicated clearly that even the smaller molecules with hydroxyl group(s) gave a sucrose response and that the fewer the hydroxyl groups, the lower the electric response (Fig. 3).

Finally, since it was revealed in Fig. 3 that small molecules with hydroxyl group(s) give electric responses, cyclohexanediol and cyclopentanediol with *trans* and *cis* isomers were used for measurement using the GL1 sensor, as the simplest molecular structure similar to six-membered monosaccharides such as glucose and fructose (Fig. 4). The

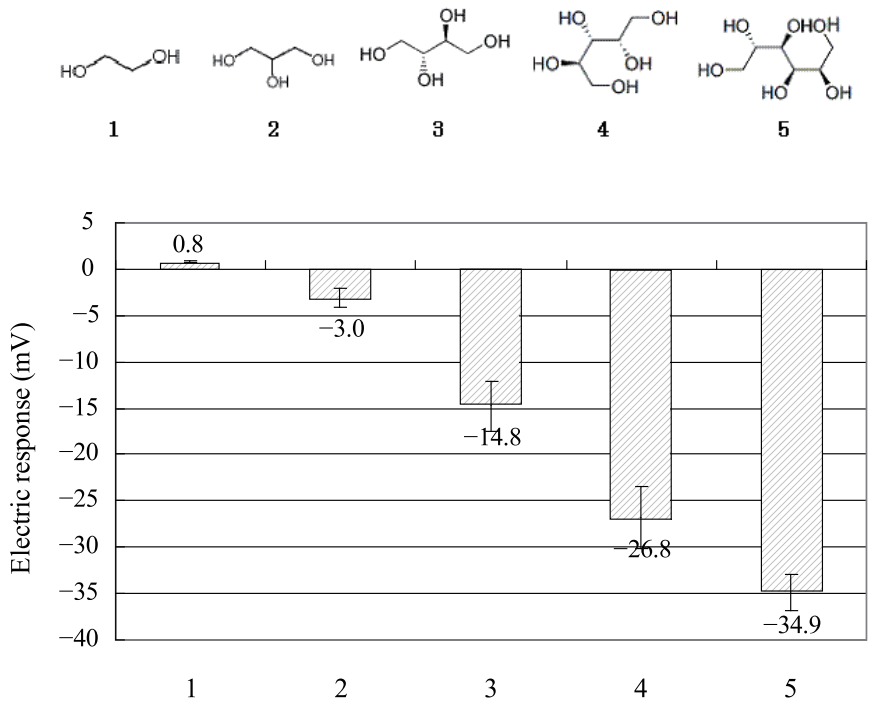
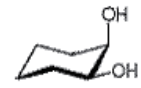
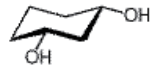


Fig. 3. Electric response of sweetness sensor GL1 to 300 mM sample solution; (1) ethylene glycol, (2) glycerol, (3) erythritol, (4) xylitol, (5) sorbitol.

trans-1,2-cyclohexanediol

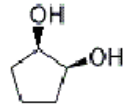


cis-1,2-cyclohexanediol

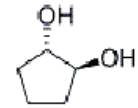


1,3-cyclohexanediol
(mixture of *cis* and *trans*)

1,4-cyclohexanediol
(mixture of *cis* and *trans*)



cis-1,2-cyclopentanediol



trans-1,2-cyclopentanediol

	Electric response (mV)	Distance of O-O (Å) between two adjacent hydroxyl groups
trans-1,2-Cyclohexanediol	–35	2.9
cis-1,2-Cyclohexanediol	–28	2.7
trans-1,2-Cyclopentanediol	–13	3.5–3.7
cis-1,2-Cyclopentanediol	–30	2.6–2.9
1,3-Cyclohexanediol	2.6	4.2–4.9
1,4-Cyclohexanediol	2.7	4.5–5.7

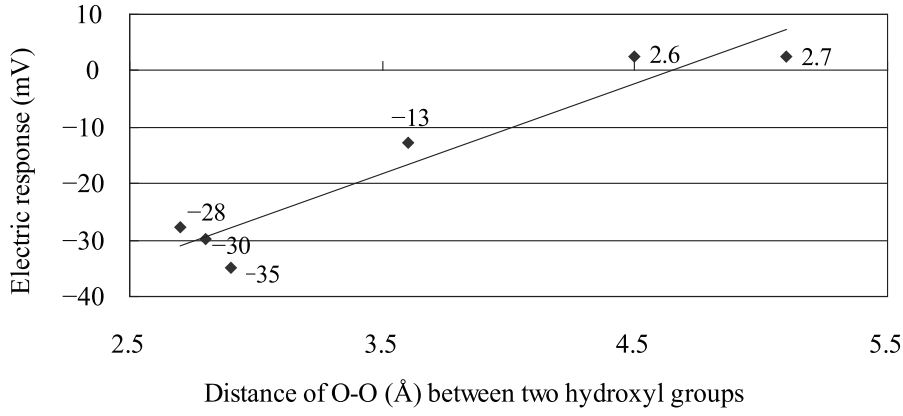


Fig. 4. Electric response of sweetness sensor GL1 to 1 M solution of cyclic diols.

merit of using them is that the effect of anomeric conversion need not be considered because they have a closed ring structure having no aldehyde groups. It was predicted that their electric responses would be lower because of the limited number of hydroxyl groups, and hence, the concentration was increased to 1 M for sample solutions, in contrast to that in the former experiments.

The results revealed that substantial electric responses were observed for both *trans*-1,2-cyclohexanediol and *cis*-1,2-cyclohexanediol, but almost zero for 1,3- or 1,4-cyclohexanediols among the cyclohexanediols (Fig. 4). This suggests that two adjacent hydroxyl groups are necessary for electric responses. Then, for cyclopentanediols, a substantial electric response was observed for *cis*-1,2-cyclopentanediol, but a lower response was observed for *trans*-1,2-cyclopentanediol. To consider the reason why the electric response from *trans*-1,2-cyclopentanediol was relatively lower although it has two adjacent hydroxyl groups, the distances between the two adjacent hydroxyl groups of each diol were checked and plotted as shown in Fig. 4. The correlation coefficient between electric responses and the distances between two adjacent hydroxyl groups was calculated as 0.96. This result suggests that the distance between the two hydroxyl groups is important for electric response; the optimum distance is approximately 3 Å.

3.2 *Interaction between sweetness sensor and sugars*

From the data above and also the data reported,[12] the electric response of the sweetness sensor to sugars may be explained by the interaction between a carboxyl group of the sensor and the two vicinal hydroxyl groups of sugar molecules. If the interaction is direct, then, it appears that it is most likely derived from hydrogen bonding. Moreover, it seems that this interaction itself must occur on the surface of the lipid/polymer membrane of the sweetness sensor, that is, oil/water interface, like in the other examples reported.[13,14] However, even if such an interaction itself may be possible there, the reason why an electric potential change is observed in the sweetness sensor from sugars, which are nonelectrolytes, is still unknown. On the other hand, if the interaction is indirect, then, there may be some mediating substances between the carboxyl group and the two vicinal hydroxyl groups. If the mediating substances are electrolytes, the reason for the electric potential change may be explainable. Further analysis is still needed for this study.

4. Conclusions

To compare the electric responses of the sweetness sensor with the chemical structure of samples, various sugars or derivatives were measured with the sweetness sensor. The results demonstrated that the presence of two adjacent hydroxyl groups in a sugar molecule is important and that the optimum distance between the two hydroxyl groups is approximately 3 Å for electric response. Possible interactions between the sensor and sugars were proposed.

Acknowledgements

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