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Discrimination of Taste Qualities Using Static and Dynamic Responses of Multichannel Taste Sensor

Kiyoshi Toko, Yuji Nakagawa, Mutsunori Obata[1] and Takeki Yahiro[2]

Department of Electronics, Faculty of Engineering, Kyushu University 36, Fukuoka 812, Japan
[1] Oita Industrial Research Institute, Oita 879-76, Japan
[2] Tokai University Junior College, Fukuoka 811-41, Japan

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Static and dynamic electric responses to various kinds of taste substances in lipid/polymer membranes were investigated. The changes in membrane electric potentials were analyzed using multivariate analyses such as multiple regression and cluster analyses. Taste qualities were quantified and classified into clusters according to the kinds of electrochemical and hydrophobic interactions between lipid/polymer membranes and taste substances. The clustering process of taste qualities in static responses was different from that in dynamic responses. Therefore, it was concluded that static and dynamic electric responses in lipid/polymer membranes contain different taste information. This finding facilitates development of a novel taste sensor utilizing static and dynamic responses.

1. Introduction

When humans consume various foods and drinks, they can quickly distinguish taste qualities such as sweetness, bitterness, sourness, saltiness and umami. Umami is the fifth independent taste quality, which is elicited by monosodium glutamate (MSG) contained in seaweed or disodium inosinate in meat and fish.[1] Dynamic as well as static responses are utilized to distinguish such qualities, which entail interaction of taste substances with gustatory cell membranes. The response velocity at the reception level may also play an important role.[2]

A multichannel taste sensor using lipid/polymer membranes has been used to discriminate and characterize taste qualities using static (or almost steady) patterns of electric potentials.[3-6] In order to mimic the mechanism of taste recognition in biological systems, it is advantageous to utilize temporal information contained in dynamic as well as static responses of lipid/polymer membranes.

The aim of this paper is to discriminate taste substances using dynamic as well as static responses. First, multiple regression analysis was applied to electric potential changes in eight different lipid/polymer membranes of a multichannel electrode with the concentrations of taste solutions measured at the steady state. The similarity between taste qualities was defined by vectors calculated from the coefficients of the multiple regression analysis; the cluster analysis of taste qualities was performed using this similarity value.

For the dynamic response, electric potentials in the taste solution were measured continuously after the taste substance was added to 1 mM KCl solution. The response of each taste sensor channel was classified into one of three categories according to the shape of the response curve, and each taste substance was characterized using this classification. The cluster analysis was performed using another similarity value between two kinds of substances defined by the sign assigned to the initial response direction of each channel.

It was found that the clusters in the static response reflect differences of electrolytes and substances adsorptive to the membranes. For the clustering process in the dynamic response, clusters were formed one by one from a cluster of sucrose and NaCl; chemical substances showing weaker interactions with lipid/polymer membranes clustered first, followed by substances showing stronger interactions which adhered to the already formed cluster. These results indicate that static and dynamic responses contain different taste information.

2. Materials and Methods

The lipid/polymer membranes used as receptors for taste substances were transparent, soft films about 200 μm thick, made from a mixture of lipid, PVC (polyvinylchloride) and DOPP (dioctylphenylphosphonate). Each lipid was mixed in a test tube containing PVC and DOPP which were dissolved in tetrahydrofuran. The mixture was then dried in a glass plate that was set on a hot plate where the temperature was held at about 30°C.

The taste sensor used here was similar to that previously reported.[3-6] For the dynamic response measurement, each of the eight kinds of lipid/polymer membranes was fitted on an electrode. For the static response measurement, the same eight membranes were fitted in the electrode; i.e., the measurement was performed using each electrode with each membrane and repeated for eight kinds of membranes. Lipid species used are abbreviated as follows: dioctyl phosphate, DOP; trioctyl methylammonium chloride, T; decyl alcohol, DA; oleic acid, OA; oleyl amine, OAm. Lipid/polymer membranes such as DOP:T = 9:1, 5:5, 3:7, which imply the molar concentrations of two lipids, were also used.

The electric potential across the membrane was detected by Ag/AgCl wire in a cavity filled with 100 mM KCl solution and Ag/AgCl wire in a reference electrode filled with saturated KCl. The electrode was put into the taste solution to be measured, which was

contained in a 250 ml beaker, without stirring. The electric membrane potentials were converted to digital code by a digital voltmeter through a high-input impedance amplifier. The data sampling processes were controlled using a personal computer.

We used 1 mM KCl solution as the standard for both static and dynamic response measurements. To obtain the static response, we measured the electric potentials of the multichannel electrode in the 1 mM KCl solution, adding the taste solution sequentially at 5 min intervals. As is revealed by the time course of electric potentials with application of taste substances, this interval is sufficient to bring the system close to the steady state (see Fig. 4). Of course, it cannot be regarded as sufficiently long for chemical substances such as quinine, which is strongly and slowly adsorbed on the membrane.[3,6,7] However, the purpose of this measurement is to obtain the values of response electric potentials at a definite time. If the measurement is performed regularly and membranes are well rinsed, precise, reliable measurements can be made, as exemplified by the small standard deviations reported previously.[4,5,8–10]

For the dynamic response measurement, changes in electric potentials of the taste sensor electrode with time were measured continuously. In most cases, the electric potential change in 1 mM KCl solution was within about ±0.5 mV after 1 mM KCl solution had been measured for about 60 s, as mentioned above and shown previously.[4,5,8–10] Then we quickly put the electrode into the taste solution where the time course of electric potentials was measured for 400 s. The measurement was performed three times for each chemical substance.

We used five typical taste-producing chemical substances,[1,2,11,12] i.e., NaCl for saltiness, HCl for sourness, quinine for bitterness, sucrose for sweetness and MSG for umami. Multiple regression and cluster analyses were applied to the data measured.[13,14]

3. Results

3.1 *Static response*

Figure 1 shows static responses of four membranes (DOP, 5:5, T, OAm) of the eight to the five typical taste-producing chemical substances. The starting origin (0 mV) is chosen to be the response electric potential at 1 mM KCl with no added taste-producing chemical substance. Standard deviations in the same eight membranes were small: *e.g.*, in the membrane of DOP:T = 5:5, the deviations were 0.18, 0.43, 0.56, 0.77, 0.36, 0.76, 0.89, 0.96 mV for 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0, 10.0 mM quinine, respectively. The response electric potential is different for different chemical substances in each membrane, and furthermore is different in other membranes. This implies that taste quality is distinguishable using the response pattern constructed from response electric potentials of several membranes, as previously shown.[3–10] In general, response to electrolytes such as NaCl, HCl and MSG is much larger than that to nonelectrolytes such as sucrose, because the membrane electric potential can be easily changed by electrolytes due to the changes in surface electric charge density and width of the electric double layer formed near the surface.

We determined the multiple regression coefficients by performing multiple regression

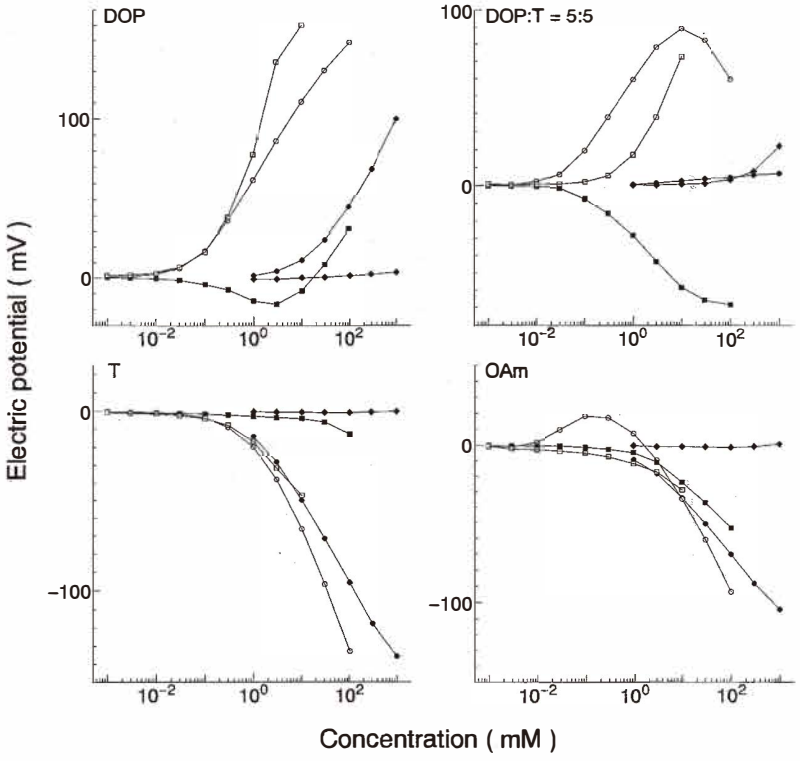

Fig. 1. Static responses of four kinds of lipid/polymer membranes to the five typical taste-producing substances, averaged over the same eight membranes. The ordinate and abscissa represent the electric potential (mV) and the concentration (mM) of taste-producing substances, respectively. ●, NaCl; ○, HCl; ■, MSG; □, quinine; ◆, sucrose.

analysis, as shown in Fig. 2, using the following linear equation:

$$\text{estimated concentration (log [mM])} = \sum_i a_{ij} V_{ij}\,, \tag{1}$$

where i denotes each channel (DOP, 5:5, T, OAm and so on), j is the chemical substance (salty, sour, bitter, sweet and umami), and V_{ij} is the response electric potential of channel i for chemical substance j. There may be a good way to use nonlinear multiple regression analysis for expressing such complex curves in Fig. 1, however, the increasing number of explanatory variables seems to be unfavorable, because the number of data is 10 or so in the present case. Therefore, we tried to express the response curves quantitatively using linear multiple regression analysis.

In the case of the 4 channels of DOP, 5:5, T and OAm membranes, the correlation

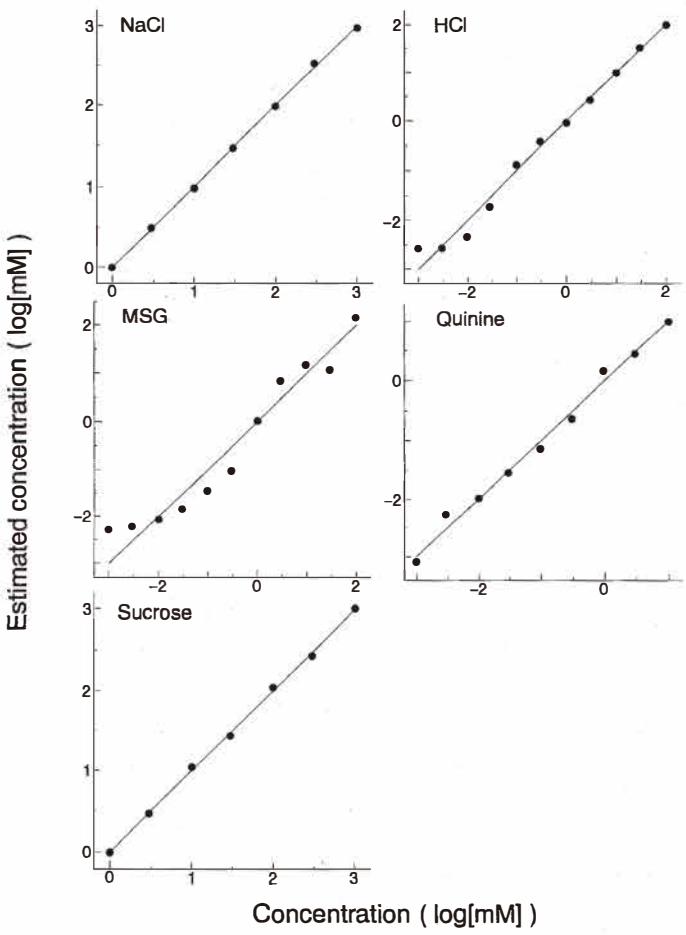

Fig. 2. Result of multiple regression analysis for the five typical taste-producing substances using four channels of DOP, 5:5, T and OAm membranes. The ordinate represents the concentration (log[mM]) calculated from the electric potential pattern, the abscissa the actual concentration (log[mM]). The solid line drawn at about a 45° angle implies the ideal correlation.

coefficients were high for all the taste qualities overall. The concentrations of NaCl, HCl, quinine and sucrose were expressed quantitatively using the four sensor outputs. The quantitative estimate of MSG concentration is not very accurate because responses to sodium ions and glutamate ions appeared simultaneously,[7] and their contribution to the response was different at low and high concentrations, as seen in the DOP membrane in Fig. 1. A set of multiple regression coefficients a_{ij} are expected to represent the characteristics of the taste substances here. Thus, we can define the degree of similarity S_{AB}^{static}

between chemical substances as:

$$S_{AB}^{\text{static}} = \frac{\boldsymbol{A} \cdot \boldsymbol{B}}{|\boldsymbol{A}|\,|\boldsymbol{B}|}, \tag{2}$$

with

$$\boldsymbol{A}, \boldsymbol{B} = (a_{1j}, a_{2j}, a_{3j}, a_{4j}), \tag{3}$$

where $\boldsymbol{A}$ and $\boldsymbol{B}$ are vectors, each of which is composed of a set of the multiple regression coefficients a_{ij} corresponding to a chemical substance j. Table 1 shows the degree of similarity S_{AB}^{static} between taste-producing chemical substances.

Figure 3 shows the result of the cluster analysis based on S_{AB}^{static}, where the attached values imply the similarity between clusters. The similarity value between one cluster and one chemical substance (or another cluster) was calculated by averaging similarity values between two chemical substances, one of which belongs to the cluster; i.e., the similarity values were obtained for a center of balance of the cluster. For the similarity value, *e.g.*, between sucrose and one cluster made of MSG and quinine, we get (0.459 + 0.677) / 2 = 0.568 because the similarity value between sucrose and MSG is 0.459 from Table 1 and that between sucrose and quinine is 0.677.

Table 1
The similarity values between taste-producing substances.

	NaCl	MSG	Quinine	Sucrose
HCl	0.991	0.385	0.014	0.308
NaCl		0.266	0.099	0.368
MSG			0.758	0.459
Quinine				0.677

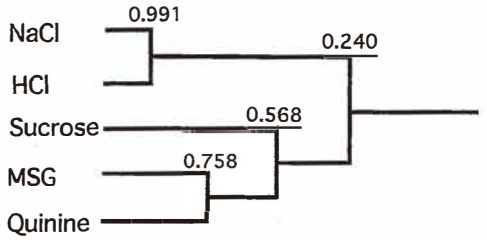

Fig. 3. Result of the cluster analysis based on the similarity between the five typical taste-producing substances. The attached values are the similarity values given in Table 1 and those between clusters calculated using these similarity values.

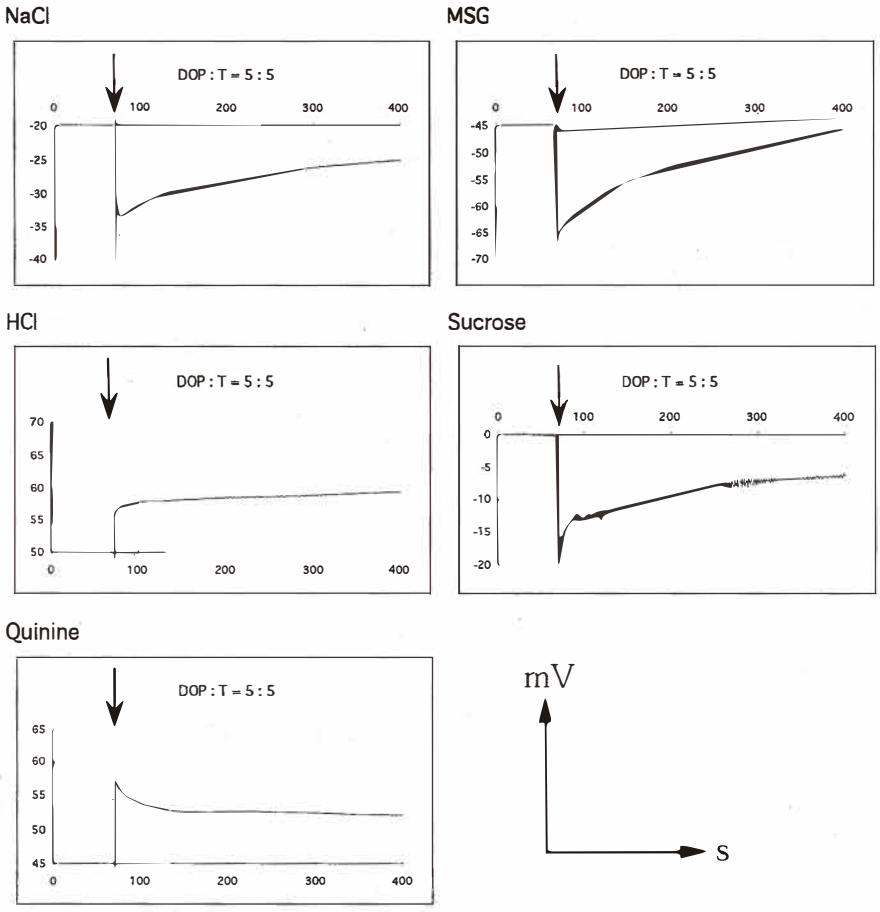

Fig. 4. Time course of electric potential changes in the DOP:T = 5:5 membrane for the five typical taste-producing substances. The ordinate represents the electric potential (mV) and the abscissa represents time (s). An arrow represents the time at which the electrode was immersed in the taste solution. Each response is averaged over three measurements.

3.2 *Dynamic response*

Figure 4 shows examples of the dynamic responses of electric potentials of the membrane of DOP:T = 5:5 to the five typical taste-producing chemical substances. The concentrations used were 30, 3, 3, 100 and 100 mM for NaCl, HCl, quinine, MSG and sucrose, respectively. The response curves were averaged over three independent measurements. Since the response electric potential for the taste solution is shown directly in Fig. 4, the values are different from those in Fig. 1, where the difference in the electric potential between 1 mM KCl and taste solution is shown. The response to HCl is fastest

among the taste-producing chemical substances studied here. All responses to taste substances tended to be faster with increasing concentration (data not shown).

The above dynamic responses can be classified into three categories. For the DOP:T = 5:5 membrane, electric potentials increased with time in the cases of NaCl, MSG and sucrose, decreased in the case of quinine, and after an initial abrupt change, changed only slightly in the case of HCl. We assign the first, second and third cases to 1, –1 and 0, respectively. Table 2 shows the classification of dynamic responses of all the channels. The signs of channels for chemical substance A, B are represented as follows:

$$A_{ij}, B_{ij} = \begin{cases} 1 \\ 0\,, \\ -1 \end{cases} \quad i = 1, \cdots, 8. \tag{4}$$

In this case, it is natural to introduce the similarity S_{AB}^{dynamic} by

$$S_{AB}^{\text{dynamic}} = \sum_{i=1}^{8} \delta_{A_i B_i}\,, \tag{5}$$

Table 2
The result of classification into three categories (1, –1 and 0). The meanings of 1, –1 and 0 are explained in the text.

	DOP	9:1	5:5	3:7	T	DA	OA	OAm
NaCl	–1	0	1	1	0	–1	–1	0
MSG	0	1	1	1	1	1	1	1
HCl	0	0	0	0	1	1	0	0
Sucrose	0	0	1	1	0	0	0	0
Quinine	1	–1	–1	–1	–1	–1	–1	–1

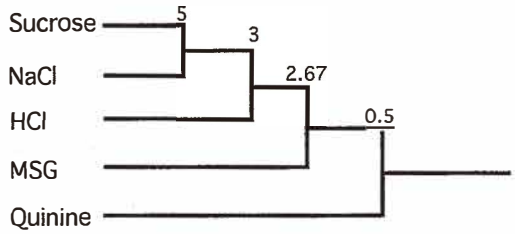

Fig. 5. Result of cluster analysis using the data shown in Table 2. The attached values are the similarity values and those between a cluster and a taste-producing substance calculated from these similarity values.

where δ is Kronecker's δ, which is unity if the signs of A_i and B_i are the same (if not, it becomes 0).

Figure 5 shows the result of cluster analysis based on the similarity S_{AB}^{dynamic} using the data in Table 2. We made the same cluster analysis as that for S_{AB}^{static} in Fig. 3. We can see that a cluster grows by absorbing one chemical substance after another in the order given.

4. Discussion

The concentration of each taste substance can be quantified using the results shown in Fig. 2, which are based on the steady-state measurement. As already shown in previous works,[4,15] the taste interactions occurring in, *e.g.*, sourness and saltiness, can also be expressed by the situation in which two kinds of taste substances coexist in solution. Using this reasoning, the multichannel taste sensor with lipid/polymer membranes can be applied to many kinds of foodstuffs such as beer,[6] sake,[8,16] milk,[9] coffee[10] and mineral water.[17]

The result of the cluster analysis shown in Fig. 3 obtained from the static response shows that taste qualities are loosely classified into two groups comprised of strong electrolytes (NaCl, HCl) and chemical substances (MSG, quinine) which are adsorbed onto the lipid/polymer membranes; i.e., salty and sour tastes are very different from those of umami and bitterness. The dynamic response result shown in Fig. 5 contrasts sharply with that in Fig. 3. First, one cluster is formed from sucrose (sweetness) and NaCl (saltiness). Subsequently, it grows by absorbing HCl (sourness). The next taste to be absorbed in the process is MSG (umami), and finally, quinine (bitterness).

In general, quinine is adsorbed onto the lipid/polymer membranes by its hydrophobic part.[18] MSG is adsorbed via a similar mechanism.[7] Hydrogen ions of HCl, on the other hand, can be bound to a dissociation group of lipids. Sucrose and NaCl may affect the lipid/polymer membranes indirectly by changes in the electric double layer formed at the membrane/water interface. Taking this fact into account, the result shown in Fig. 5 suggests that chemical substances which show weak interaction with lipid/polymer membranes are first gathered into one cluster, and then the chemical substance which shows the second strongest interaction with the membranes is absorbed on the cluster. This process is repeated in order to form one large cluster comprised of all five taste qualities.

The two results shown in Figs. 3 and 5 imply that the static response of lipid/polymer membranes contains taste information that is different from the dynamic response. Therefore, we can construct a novel taste-sensing system by utilizing these static and dynamic responses. In addition, this study may help clarify the biological reception mechanism of taste-producing substances.

In the present paper, the dynamic responses were classified into three categories qualitatively. Using this method, however, it is difficult to quantify the taste intensities. Quantification of taste might be possible using the time constant, which is the inverse of the velocity of electric potential change, because its characteristics should be affected by the interaction of taste substances with lipid/polymer membranes. In fact, the time constant differs greatly among the taste substances shown in Fig. 4, as was demonstrated in an artificial lipid membrane.[19]

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