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Measurement of Hydrophobicity of Amino Acids Using a Multichannel Taste Sensor

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A multichannel taste sensor with lipid/polymer membranes was used to study the hydrophobicity of amino acids, which exhibit complex taste compositions, *e.g.*, bitterness and sweetness. A strong correlation was found between sensor output and hydrophobicity. This implies that the taste sensor can quantify the hydrophobicity of chemical substances, which partly determines the taste.

1. Introduction

Amino acids have received much attention because they exhibit complex taste compositions.[1–4] Monosodium L-aspartate shows both umami and salty tastes, while L-lysine monohydrochloride shows three kinds of taste: bitter, sweet and umami. L-Valine and L-methionine taste strongly bitter and mildly sweet.

The mechanism of taste production by amino acids has not been clarified. However, one interesting fact was pointed out regarding the relationship between hydrophobicity and bitterness of amino acids; i.e., amino acids show stronger bitterness with increasing hydrophobicity.[4]

A recent study[5] using a multichannel taste sensor with lipid/polymer membranes showed that the taste of amino acids can be classified into five groups: those showing sweet, bitter, sour and umami tastes and an intermediate group showing both sweet and bitter tastes; this result is consistent with the result of sensory evaluation.[1–3]

In the present study, the relationship between hydrophobicity of amino acids and sensor output was studied on the basis of the above facts. As a result, a strong correlation is found between them, and the response mechanism of the taste sensor with lipid/polymer membranes is discussed.

2. Materials and Methods

The eight lipid materials used in the multichannel taste sensor are listed in Table 1. Lipid/polymer membranes were made according to the method reported previously.[5,6] The electrode and the measurement system were the same as before.[5,7] The electric potential difference between the multichannel electrode and a reference electrode (Ag/AgCl with saturated KCl) was measured. The multichannel electrode was connected to an 8-channel scanner through high-input impedance amplifiers. Selected electric signals from the sensor were digitized and processed by a computer.

Eleven kinds of amino acids were provided by Ajinomoto Co., Inc. and were dissolved in water containing 1 mM KCl prior to the experiments. The response electric potentials were measured by setting the response to 1 mM KCl solution as the origin.

Table 2 gives data on the taste and hydrophobicity of amino acids as reported previously.[1–4] The values listed are the free energy change Δf calculated from the solubility data of individual amino acids. It was suggested that amino acids are not bitter if $\Delta f < 1300$, while they are bitter if $\Delta f > 1400$. A positive value of Δf means that the amino acid is insoluble in water from the viewpoint of thermodynamics. Thus, amino acids with large Δf are hardly soluble in water. In other words, amino acids with large Δf are hydrophobic, whereas those with small Δf are hydrophilic. Therefore, we call Δf "hydrophobicity" hereafter.

3. Results and Discussion

Figure 1 shows response electric potential patterns for eleven kinds of amino acids: 56 mM L-alanine, 116 mM monosodium L-aspartate, 2 mM L-glutamic acid, 133 mM glycine,

Table 1
Lipid materials. Lipids were used separately in two kinds of electrodes, A and B. Membranes of channels 4, 5 and 6 were composed of two kinds of lipids, i.e., dioctyl phosphate ($2C_8POOH$, abbreviated as C) and trioctyl methyl ammonium chloride (TOMA, abbreviated as T), with the ratios shown.

	Channel no.	Lipid
A	1	Decyl alcohol (DA)
	2	Oleic acid (OA)
	3	Dioctyl phosphate (C)
	4	C/T = 9/1
B	5	C/T = 5/5
	6	C/T = 3/7
	7	Trioctyl methyl ammonium chloride (T)
	8	Oleyl amine

Table 2
Data on taste[1–3] and hydrophobicity[4] of amino acids. The second term in the taste expression implies weaker taste intensity than the first term.

Amino acid	Taste	Hydrophobicity (cal/mol)
L-Alanine (Ala)	Sweet	730
Monosodium L-aspartate (Asp. Na)	Umami + Salty	540
L-Glutamic acid (Glu)	Sour + Umami	550
Glycine (Gly)	Sweet	0
L-Isoleucine (Ile)	Bitter	2970
L-Lysine monohydrochloride (Lys. HCl)	Bitter + Sweet	1500
L-Methionine (Met)	Bitter + Sweet	1300
L-Phenylalanine (Phe)	Bitter	2650
L-Threonine (Thr)	Sweet	440
L-Tryptophan (Try)	Bitter	3000
L-Valine (Val)	Bitter + Sweet	1690

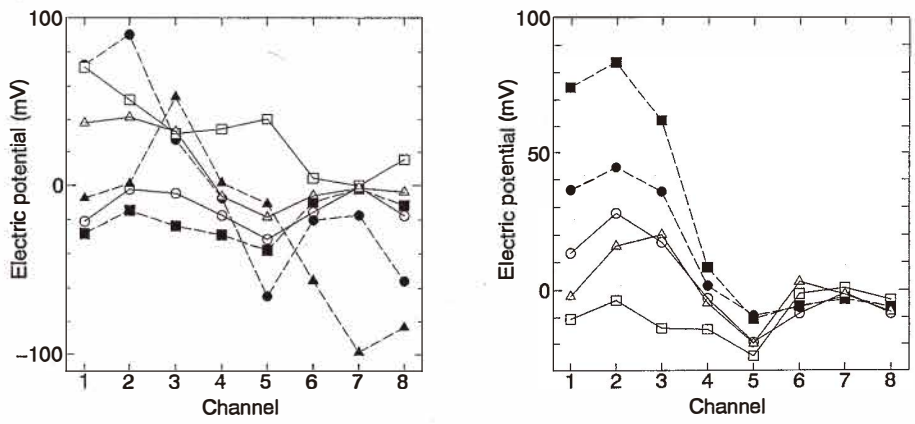

Fig. 1. Response electric potential patterns for amino acids. Ala (○), Asp. Na (●), Glu (□), Gly (■), Ile (△) and Lys. HCl (▲) in (a); and Met (○), Phe (●), Thr (□), Try (■) and Val (△) in (b).

46 mM L-isoleucine, 164 mM L-lysine monohydrochloride, 20 mM L-methionine, 18 mM L-phenylalanine, 168 mM L-threonine, 15 mM L-tryptophan and 68 mM L-valine, where the concentrations were the same as those in human sensory evaluations.[1] A large variation in the patterns is observed, and the amino acids are easily discriminated because of the small

standard deviation of 1–3 mV, as previously shown.[5]

For bitter substances such as Ile, Phe and Try, channels 1 to 3 show large values, whereas almost all the channels show small values for sweet substances such as Ala, Gly and Thr. The response patterns for Lys. HCl, Met and Val showing both bitter and sweet tastes are located between the above bitter and sweet substances, although the response to Cl^- of Lys. HCl appears in channels 6 to 8. One of the sour amino acids, Glu, increases the response potentials of channels 1 to 5. Asp. Na, which shows umami taste, has a response pattern very different from those of other amino acids.

As a next step, the relationship between sensor output in Fig. 1 and hydrophobicity of amino acids in Table 2 was studied. For this purpose, we used a kind of stepwise method used in multiregression analysis.[8] The method is as follows: multiregression analysis was applied to determine the hydrophobicity Δf (objective variable) using eight explanatory variables of response electric potentials of all eight channels. The multiregression coefficient adjusted to the degree of freedom $\hat{R}$ was calculated to give a quantitative agreement between objective and explanatory variables. Next, the number of explanatory variables was reduced to seven by omitting one of them, and then $\hat{R}$ was calculated for the resulting eight cases in the same way. The same procedure was repeated with the number of explanatory variables being reduced each time. During this process, one set of explanatory variables was adopted when $\hat{R}$ was a maximum, because the objective variable was considered to be expressed most quantitatively in this case.

The result is shown in Fig. 2, where we can see a good correlation between sensor output and hydrophobicity. The multiregression coefficient adjusted to the degree of freedom $\hat{R}$ was 0.977, which seemed to be sufficiently high for explaining the objective variable, i.e., the hydrophobicity of amino acids. The selected set of five channels was composed of ch. 1 (DA), ch. 2 (OA), ch. 3 ($2C_8$POOH), ch. 5 (5/5) and ch. 7 (TOMA). This result is satisfactory; in a previous study,[5] channels 1, 2 and 3 contributed significantly to the first principal component (PC1), in which the bitter taste of amino acids was distinguished from the sweet taste, whereas channels 6 and 8 hardly contributed to it.

When hydrophobicity was expressed by only two channel outputs as a trial, $\hat{R}$ became 0.723, which also seemed high, using $2C_8$POOH and TOMA membranes. The result is very interesting, because these two membranes are negatively and positively charged, respectively. Hydrophobicity, which may be related to bitter taste,[4] can therefore be quantified using two lipid/polymer membranes with different characteristics.

The reason why the taste of amino acids was discriminated by the taste sensor[5] is partly due to the strong correlation of sensor outputs with the hydrophobicity of amino acids, which may be a global property of molecules. Grouping of the tastes of amino acids into bitter, sweet, sour and umami may reflect this kind of property which is determined from the hydrophobicity and hydrophilicity of molecules. Molecular recognition by specific proteins of the biological membrane may also have an effect on taste reception.[9] During the initial reception of chemical substances, global selectivity, which originates from hydrophobic and hydrophilic interactions between the biological membrane and chemical substances, is considered to be very important. The taste of foodstuffs can be satisfactorily discriminated and quantified due to the global selectivity ability of the lipid/polymer membranes used in the taste sensor. For example, the titratable acidity in sake was

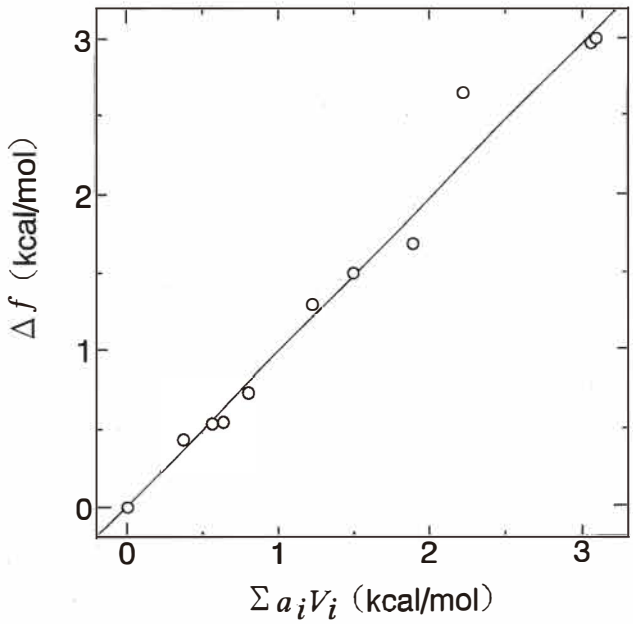

Fig. 2. Relationship between hydrophobicity of amino acids and sensor outputs. The abscissa represents the multiregression linear equation with V_i denoting the output of channel i (= 1, 2, 3, 5, 7) and a_i the multiregression coefficient, and the ordinate represents the data on hydrophobicity of amino acids.[4]

quantified using the taste sensor[10] because of its ability to detect amino acids and organic acids. High sensitivity of the taste sensor to protein denaturation in milk[11] may be related to the appearance of charged groups in the aqueous phase, which affects the response electric potentials of the lipid/polymer membranes of the taste sensor.

We propose two approaches to the further development of taste sensors. One is the active utilization of this kind of global selectivity, which will contribute significantly to automated control and monitoring of food production in various fields.[10–12] The sensor can also be applied to real-time, rapid detection of water pollution. After the pollutant is rapidly detected using the taste sensor, we can analyze the molecules that pollute the water in detail using conventional apparatuses for chemical analyses, which are inadequate for real-time detection. Of course, the long-term stability of lipid/polymer membranes used in the taste sensor is very high, as shown.[10–12] Another approach is the incorporation of molecular recognition ability in the taste sensor by using a specific lipid or protein. In this case, multivariate analysis used in chemical sensing[13] may have to be conducted in order to obtain useful information, because taste is essentially produced by many chemical components.

There are several levels of selectivity or recognition in biological systems. It is very important to classify chemical substances into five kinds of tastes in taste reception, because these tastes have individual physiological meanings. The present taste sensor using lipid/polymer membranes can provide information on this kind of molecular difference. As shown here, studies using the taste sensor will help clarify the mechanism of taste reception.

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