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Analysis of Tastes of Amino Acids Using Surface-Polarity Controlled Sensors

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The tastes of amino acids were studied using a method for detecting the change in the interaction between an electrode surface and amino acids in aqueous solution. The electrode impedance showed different patterns of potential profiles for amino acids with different taste qualities, while it showed similar patterns for amino acids with similar tastes. Amino acids may be classified into four groups according to their tastes based on sensor outputs. The sensor response correlated well with human sensitivities and showed higher sensitivity than human sensation. This method can provide useful transducers for the development of sensors to analyze taste qualitatively and quantitatively.

1. Introduction

The measurement of taste with artificial sensors is an essential labor-saving method for quality control in the food industry; however, the development of a taste sensor is not easy. Physical sensors have conventionally dealt with a single input such as light or sound; hence they are relatively easy to construct. Taste sensors have to deal with a large number of inputs, i.e., chemicals, and the difficulty increases with the number of inputs. Fabrication of such sensors is very difficult. However, human taste sensation is roughly classified into only five basic types: sour, salty, bitter, sweet and umami.[1,2] Hence taste sensors would be sufficient if they could discriminate among the five tastes qualitatively and quantitatively.

One way to realize taste sensors is to utilize materials similar to those in biological systems as transducers. A recently developed chemical sensor utilizes lipid membranes to

transform taste information into signals that are electric potential changes;[3–5] this sensor is able to measure both the quality and the intensity of the taste. It can be used to detect taste in a manner similar to the human gustatory sensation so that similar patterns are obtained for similar taste qualities and a sensitivity similar to that of humans is realized. Thus, the taste sensor has been applied to many kinds of foodstuffs.[6–10] The taste of foodstuffs can be examined quantitatively using the taste sensor which provides an objective scale for human sensory expressions.

Here we developed a taste sensor by an electrochemical method for controling interface potentials. Electrode surface polarization was controlled with positive, negative and neutral electric potentials. This sensor can detect neutral substances which are difficult to discriminate by electrochemical methods. Actually, the lipid membrane sensor cannot detect neutral substances with high sensitivity. This sensor used the essential detecting mechanism of the lipid membrane sensor; both polarity of the surface and interaction with chemicals are very important in sensing the tastes. To a great extent, it was possible to detect odor molecules or environmental pollutants dissolved in water.[11]

From the standpoint of taste sensor development, analysis of tastes of amino acids is very important because each amino acid elicits a complicated mixed taste. Ninomiya *et al.* investigated taste intensity and quality of various amino acids in detail by sensory panel tests.[12] Amino acids are generally classified into several groups that each correspond to a characteristic taste. In this study, we applied this sensor to amino acids and evaluated their taste according to sensor outputs.

2. Materials and Methods

2.1 *Taste substances and experimental setup*

All amino acids used in the experiments were L-type and employed without further purification. Prior to the experiments all substances were dissolved in water containing 10 mM KCl.

Figure 1(a) is a simplified diagram showing the setup of the experimental equipment. A frequency response analyzer (FRA, NF Electronics Instruments 5020) was used to measure the electrode surface impedance. A computer (Apple Macintosh) was connected to the equipment and controlled the measuring process. A Pt-electrode as a working electrode, a reference electrode composed of an Ag/AgCl wire with saturated KCl and a counter electrode of a platinum wire were all connected to a laboratory-built potentiostat.

The working electrode was made by setting the 1 mm Pt wire in an acrylic board with a 5 mm diameter hole. The hole was filled with epoxy resin. The Pt wire and epoxy resin, when dried, were polished using sandpaper and 0.1 μm alumina polishing suspension until the surface became smooth. The Pt-electrode is the most commonly used metallic solid electrode. It is resistant to oxidation, but it is not totally inert as was often assumed in early work.[13]

All three electrodes were dipped in the test solutions containing the taste substances. The potential of the Pt-electrode used as the transducer was controlled by the potentiostat. The sinusoidal current and DC voltage from the FRA were applied to the potentiostat which controlled the potential of the Pt-electrode, and the frequency response was investigated.

2.2 *Equivalent circuit of the electrode*

Figure 1(b) shows an equivalent circuit where redox reactions such as hydrogen production did not occur. The equivalent circuit was obtained by considering the roughness of the electrode surface. The equivalent circuit consists of the connection in parallel of the electrode resistance (R_r) and the electrode capacitance (C_{dl}) of the electrical double layer on the electrode surface. The term R_r is the unusual electrical resistance caused by roughness of the electrode surface, where the resistance depended on frequency f. $R_r = \alpha/f$ where α was constant. The term R_s is the solution resistance which is connected in series to the parallel connection of R_r and C_{dl}. We carried out curve fitting on the frequency response locus to obtain the resistance and capacitance of the electrode. The term R_{45} is the estimated value of R_r at $f = 45$ Hz.

2.3 *Potential profiles of standard solutions*

Figure 2 shows dependence of R_{45} and C_{dl} on the electrode potential of the Pt-electrode in 10 mM KCl as the standard solution. In this experiment, data for taste could not be obained with the addition of buffer solution instead of KCl. This implies that the taste sensor could not respond to the tastes of amino acids in buffer solution, which has constant

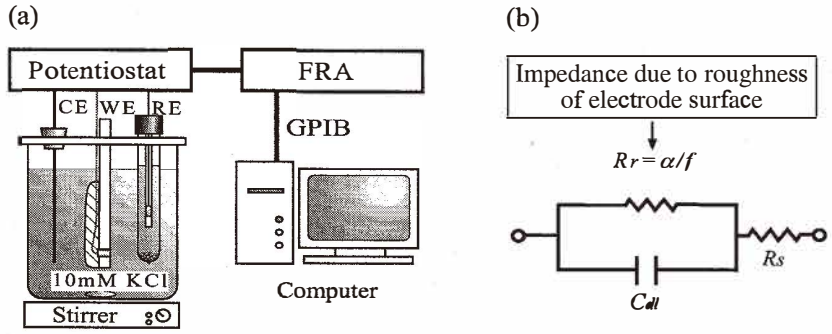

Fig. 1. Setup for the measurement of (a) the electrode impedance and (b) equivalent circuit. WE : working electrode, RE : reference electrode, CE : counter electrode.

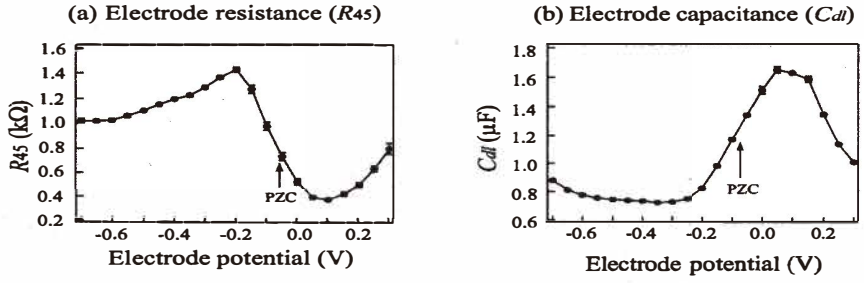

Fig. 2. Potential profiles of the Pt-electrode impedance in 10 mM KCl as the standard solution.

pH. Both R_{45} and C_{dl} depend on the electrode potentials. The electrode impedance depended mainly on the adsorption and desorption of hydrogen in the negatively charged region and oxygen adsorption/desorption in the positive region. We referred to these dependencies as potential profiles of the electrode impedance. The point of zero charge (pzc) is where the surface charge becomes zero and the surface becomes hydrophobic.

The Pt-electrode was not an electrode suitable only for surface polarization, since different surface states were present which interacted as expected with different taste substances. The changes in R_{45} and C_{dl} where taste substances were added were ΔR_{45} and ΔC_{dl}, respectively. Here we measured the changes in electrode potential and regarded the changes in the pattern as the potential profile.

2.4 *Scanning method*

A micro-signal of 0.01V in amplitude was superimposed on the electrode potential. To prevent the electrolysis of water, the electrode potential was changed over a scanning potential ranging from – 0.7 V to + 0.3 V in 0.05 V steps. The computer controlled the measurement of interface impedance between the electrode surface and the aqueous taste solution. The responses of the interface impedance were measured with setting the response to a 10 mM KCl solution as the origin.

3. Results

3.1 *Responses to amino acids*

Figure 3 shows the profiles of electric potential of electrode surface impedance for glycine (Gly), L-tryptophan (L-Trp), L-glutamic acid (L-Glu) and monosodium glutamate (MSG) which represent the sweet, bitter, sour and umami tastes of the amino acids, respectively.

The averages and standard deviations are shown for measurements made in triplicate for each substance. It is clearly seen that the resistance patterns for amino acids are different from each other. The peak around 0.1V was associated with the amino subgroup in the amino acid molecules, and could be seen for all amino acids. Electrode capacitance patterns did not differ significantly between amino acids used in the measurements.

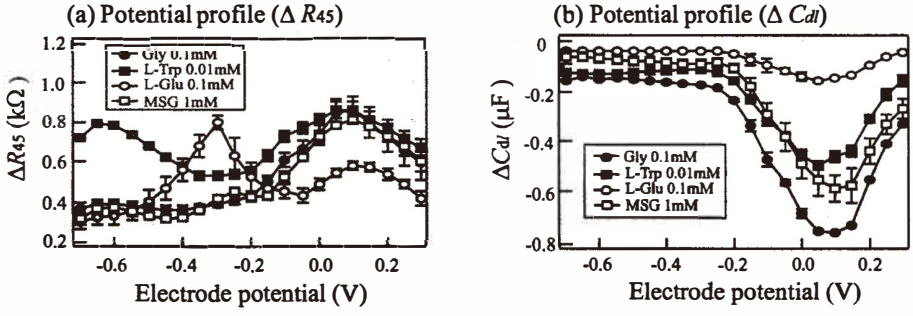

Fig. 3. Electrode potential profiles for Gly, L-Trp, L-Glu and MSG.

Consequently, we mainly discuss the taste responses of ΔR_{45}. Error bars for L-Glu were relatively large, because the pH of the solution was not precisely controlled in these experiments, and this affected the impedance significantly.

As seen in Fig. 4, different potential profiles of the sensor outputs were obtained for the different taste substances sweet, bitter, sour and umami-tasting amino acids, and similar patterns were obtained for similar taste substances. This implies that the tastes of amino acids can be measured qualitatively by this method and may be explained by interactions based on electrode polarity. Therefore, the differences among electrode potential profiles are due to the changes in the surface potential, which involve ionic concentration changes according to dissociation and adsorption of taste substances on the electrode surfaces.

The most common conventional electrochemical methods involve oxidation-reduction processes. In this study, however, the change in the interaction between the electrode surface and the chemical substances was investigated using the surface polarization in aqueous solution where no redox reaction occurs. Therefore, we obtained different potential profiles formed according to the various inherent characteristics of chemical substances.

Moreover, the changes in the interaction between the electrode surface and the chemical substances can be expected to yield information about taste, because the taste of chemicals depends mainly on the polarity of chemicals.

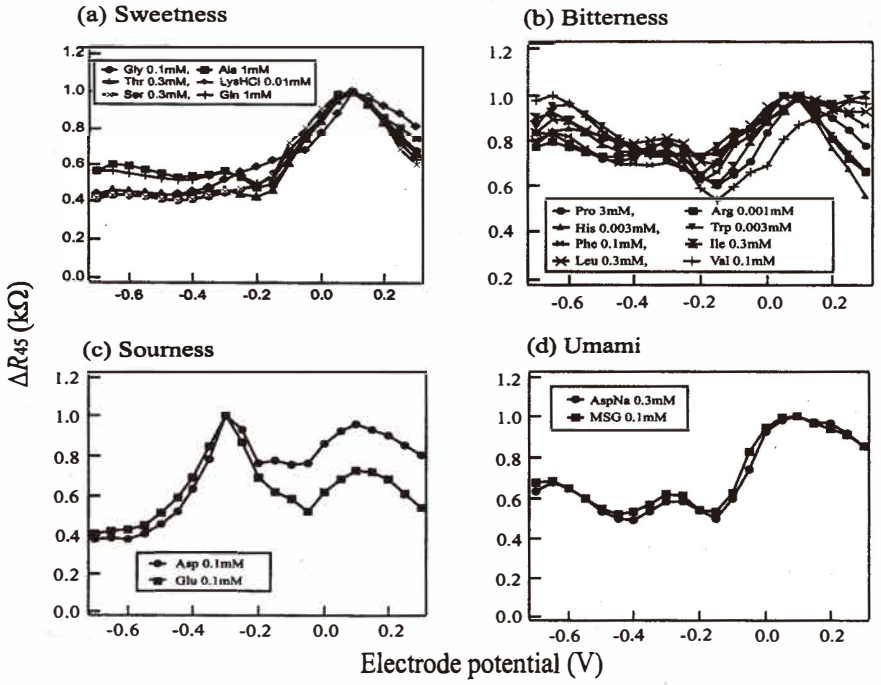

Fig. 4. Electrode potential profiles of amino acids with different taste qualities.

3.2 *Principal components analysis of sensor outputs*

Data obtained using this taste sensor (surface-polarity controlled sensor) were studied in greater detail by principal components analysis (PCA). The original data were visualized on two- or three-dimensional planes using this analysis, which is very effective in reducing the dimensional space with minimum loss of information.[14] The contribution rates to the first (PC1), the second (PC2) and the third (PC3) principal component were 63.6%, 17.2% and 9.6%, respectively. Therefore, useful discussions of PC1 and PC2 may be possible using a scatter diagram. Figure 5 shows the obtained data points plotted in a scatter diagram on the PC1 and the PC2 plane.

Sweet amino acids and bitter amino acids were located at negative and positive regions along the PC1 axis, respectively. Moreover, umami tasting amino acids were located between them. However, sour amino acids were found separately on the PC2 axis. As seen in the figure, amino acids were classified more clearly into four groups according to their taste, as expected. This PCA classification of tastes of amino acids is similar to human taste sensation for the classification of tastes of amino acids.[12]

3.3 *Cluster analysis*

PCA suggests that tastes of amino acids can be classified into four groups by evaluating the scatter diagram visually. Cluster analysis is the most commonly used term for the class of procedures that seek to separate component data into groups.[14] To identify a smaller number of groups such that elements belonging to a given group are, in some sense, more similar to each other than to elements belonging to other groups, we carried out the cluster analysis illustrated in Fig. 6.

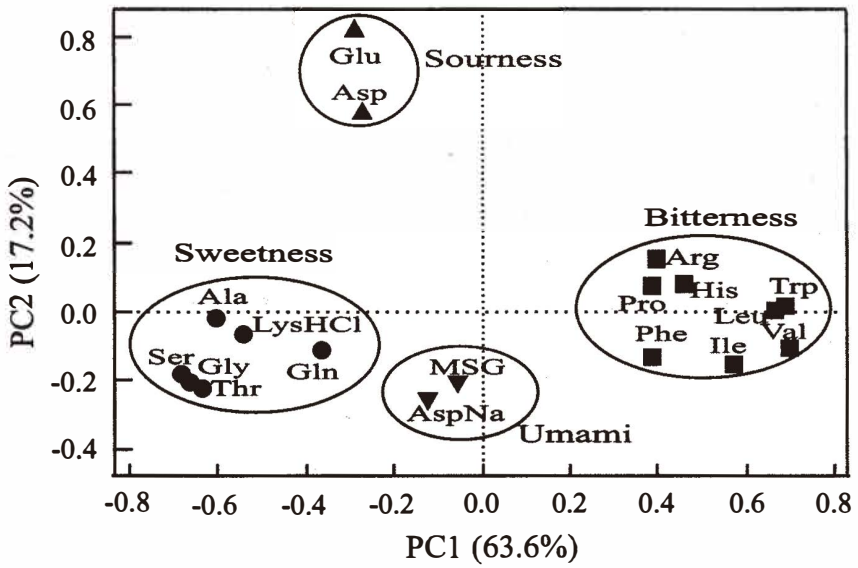

Fig. 5. Scatter diagram of principal components analysis.

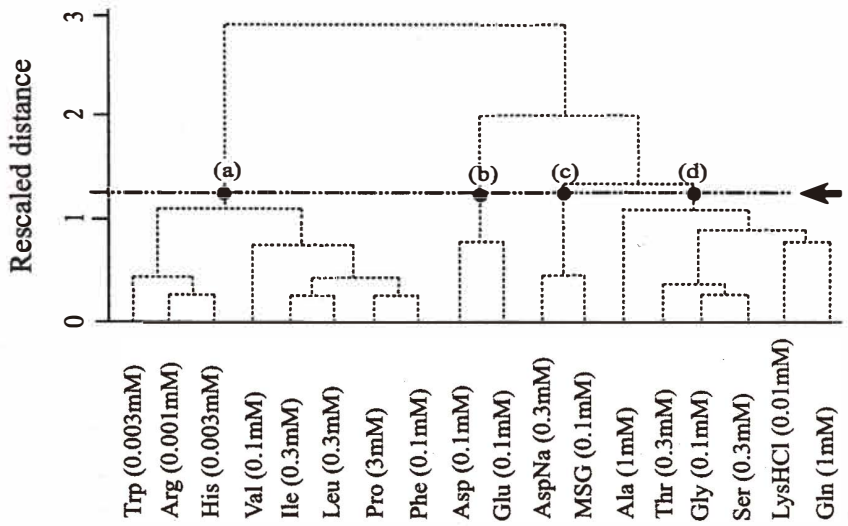

Fig. 6. Dendrogram of sensor outputs. (a) Bitterness, (b) sourness, (c) umami and (d) sweetness.

Fundamental to the use of any technique for cluster analysis is the computation of a measure of similarity or distance between the respective objects. Thus, cluster analysis intends to reduce the information on the whole set of n objects to information about, for example, h subgroups, where $h \ll n$. We selected analysis parameters to achieve compact clusters. The Ward method[14] was selected when computing distances between the groups, and we also evaluated the Euclidean distance between the objects.

As seen in Fig. 6, all of the objects could be correctly separated into four clusters based upon the taste qualities of amino acids. The sensor output indicated different cluster for amino acids which have different taste qualities, while it indicated the same clusters for amino acids which have similar tastes. This implies that the taste sensor could respond to the taste itself.

3.4 *Sensor threshold comparison with the human sense*

The threshold concentrations of the sensor for amino acids were compared with the human threshold concentrations for amino acids obtained by Schifmann *et al.*[15]

Figure 7(a) shows the relationship between the mean values of the sensor output and the scanning concentration. In this experiment, the threshold value of the sensor for each amino acid was determined according to the lowest value of the sensor response, which was the 0.2 kΩ level. In the cases of L-Gln, L-Ala and L-Pro, for example, sensor thresholds were approximately 0.005 mM, 0.04 mM and 0.05 mM respectively.

Figure 7(b) shows the relationship between the threshold values of the sensor and the human values. The solid line indicates sensor threshold values that equal human values. The dotted line is a visual guide. The sensor was 1000 times more sensitive than the human sense.

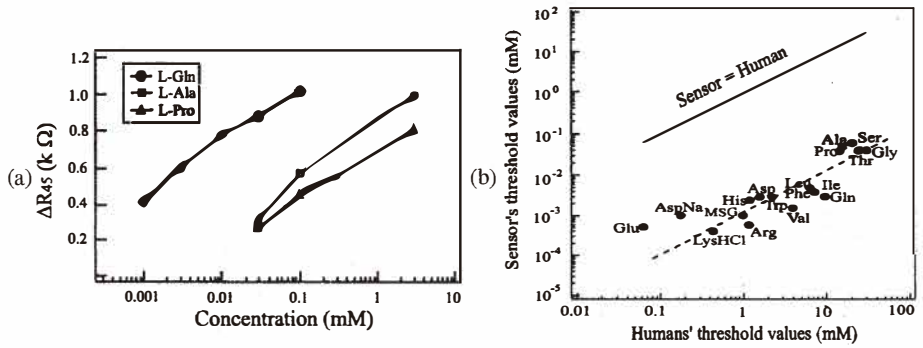

Fig. 7. Relationship between the threshold values of the sensor and humans. The threshold values of (a) the taste sensor, and (b) the sensitivity of the taste sensor.

These results show that the correlation between the sensor output and the threshold concentration of humans is linear. It also indicated the usefulness of the taste sensor for the quantitative analysis of human sensory expression. Furthermore, better agreement was obtained between sensor outputs and data from humans. The information from the sensor could be utilized to evaluate taste quality and to quantify taste intensity at the same time.

4. Discussion

Amino acids are generally considered essential for humans. Amino acids that are required for protein synthesis and cannot be synthesized by the organism must be present in the diet. Such compounds are referred to as essential amino acids. The side chains of amino acids are important, and they determine whether the amino acids are acidic, basic or neutral. They also determine the structures of the amino acids, which result in amino acids having different characteristics and tastes.

In this study, we have developed a chemical sensor by using the surface polarity control of an electrode to modify the interaction with and the sensitivity to various chemicals. Electrochemical impedance was measured at each electrode surface potential to determine the condition of the surface. This sensor could detect both electrolytes and nonelectrolytes with high sensitivity.

The potential profiles for substances with different taste qualities were significantly different, and hence each taste could be easily discriminated with high reproducibility. In contrast, the taste sensor gave similar potential profiles for substances of the same taste quality, *e.g.*, sour substances such as L-Glu and L-Asp have similar potential profiles. Umami substances such as MSG and L-AspNa also have similar potential profiles. (Fig. 4) Amino acids which have a sweet taste were located in the same region, and bitter amino acids belonged to a different region, as expected. (Fig. 5) The taste of amino acids was classified into four groups in accordance with the results of both the human sensory test and

cluster analysis. Furthermore, threshold values of amino acids detected by the sensor had good correlation with human values.

We can conclude that this sensor and its associated measurement methods respond to taste. The electrode response was more sensitive than that of humans, and it was confirmed that this method can provide useful transducers for the development of taste sensors which can be used for both quantitative and qualitative taste analysis. The taste sensor has good sensitivity, reproducibility, and a durability higher than the sense of taste of humans.

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