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Detection of Mutual Interaction between Taste Substances by Impedance Measurements of Lipid/Polymer Membranes

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Response differences between the membrane potential and the membrane impedance of lipid/polymer membranes to aqueous solutions of mixed taste substances were investigated. Thin membranes formed by dip-coating were used to measure the impedance, whereas thick films formed by the same process in a multichannel taste sensor were used to measure the electric potential. The changes in membrane impedance were significantly accelerated by the combination of two *umami* substances in a manner similar to the synergistic effect in the human sensation, whereas such acceleration did not occur in the changes in potential.

1. Introduction

Until recently, a chemical sensor for human sensations could not be realized, because it was difficult to test all the various substances that affect human chemical sensations. Taste- and odor-sensing systems with global selectivity, which is the opposite of specific selectivity in conventional sensors, have been recently developed using biomimetic technology.[1–3]

Lipids, which are major components of a biological membranes, have been identified for their sensitivity to taste substances.[4] A multichannel taste sensor with lipid/polymer membranes has been developed.[2,5] It responds to basic taste substances with global selectivity in a manner similar to the human sense.

The taste sensor is fundamentally based on the measurement of the electric potential difference across a membrane just as taste cells cause a membrane potential change as the first step in gustatory sensation. However, the sensor is less sensitive to hydrophobic or nonelectrolytic substances than the human sense.

For the purpose of detecting taste substances including nonelectrolytes, some measurement methods other than potentiometry were proposed for taste sensing using a lipid/polymer membrane.[6–9] The characteristics of the interaction between the lipid/polymer membrane and taste substances were investigated using impedance measurements,[6] surface plasmon resonance,[7] quartz oscillation[8] and semiconductor technology.[9] These attempts yielded information different from that obtained by potentiometry.

The basic response characteristics of membrane impedance when taste substances were added one at a time were reported.[6] The membrane impedance of the lipid/polymer membrane effectively responded to nonelectrolytic taste substances. There are, however, numerous taste substances in actual foods, and substances in combination can affect human chemical sensations.

In this study, the responses of membrane impedance to mixed taste substances in combination were measured and compared with those of the membrane potential. An *umami* substance was selected as one of the mixed substances. The reason is that *umami*, which was recently added as a fifth basic taste,[10] shows a synergistic effect that is a remarkable enhancement of the *umami* taste by the combination of two types of *umami* substances.[11] The synergistic effect between *umami* substances could be detected by an impedance change similar to that reported in a lipid impregnated into the pores of filter paper.[12]

2. Materials and Methods

2.1 *Lipid/polymer membranes*

The lipid/polymer hybrid membranes, which have been used as a transducer for the taste sensor, consist of synthetic lipid, polyvinyl chloride (PVC) as the forming polymer, and di-n-octylphenylphosphonate (DOPP) as the plasticizer.

The lipids are abbreviated as follows: dioctylphosphate, DOP; trioctylmethylammonium chloride, TOMA; oleyl amine, OAm; decyl alcohol, DA; oleic acid, OA. The mixtures of DOP and TOMA are named 9:1, 5:5 and 3:7, indicating the ratio of molar concentrations of the components. Each lipid, 400 mg of PVC and 0.5 ml of DOPP were mixed together in a test tube with 10 ml of tetrahydrofuran as the solvent.

A membrane to measure the electric potential was obtained by evaporation of solvent from a glass plate. The membranes formed, which were transparent and soft films ca. 200 μm thick, were placed on the surface of a multichannel electrode together with an inner solution (1 M KCl).

The 200-μm-thick membrane was not suitable to measure the impedance, because the impedance change with taste substances was very small. Therefore, a thin membrane to measure the impedance was formed by dip-coating directly on a platinum electrode 2 mm in diameter. The estimated thickness of the membrane was less than 1 μm.

2.2 *Measurement of membrane impedance*

The membrane impedance was measured using an alternating electric current source applied to the lipid/polymer membrane in the test solution. Figure 1 shows the experimental apparatus for measuring the impedance of the membrane.

A frequency response analyzer (FRA; NF electronic instruments, 5020) produced a sinusoidal voltage wave. The sinusoidal electric current converted from the voltage was applied from a counter electrode to the sensing electrode across the lipid/polymer membrane. The voltage response between the sensing electrode and the reference electrode was fed into the FRA. The FRA estimated the complex values of the membrane impedance from the input-output characteristics of gain and the phase shift.

2.3 *Measurement of membrane potential*

The membrane potential was led using the multichannel electrode with the lipid/polymer membranes and a reference electrode with an inner solution of saturated KCl. The structure of the electrodes in the test solution is as follows: Ag/AgCl wire | 1 M KCl | membrane | test solution | saturated KCl solution | Ag/AgCl wire.

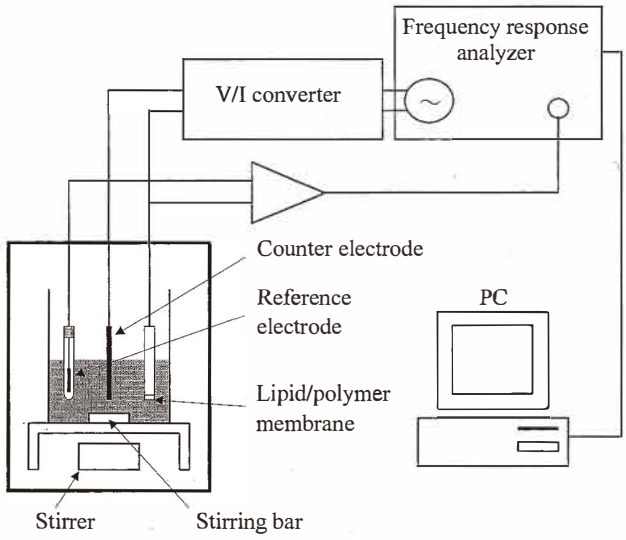

Fig. 1. Experimental apparatus to measure the impedance of the lipid/polymer membrane.

The electric potential signals from the membranes were sent to a scanner through high-input impedance amplifiers. The signals selected sequentially by the scanner were converted to a digital code by a digital voltmeter and collected in a computer.

3. Results and Discussion

3.1 *Impedance paths and equivalent circuits*

Figure 2 shows the impedance paths of the lipid/polymer membrane in a complex plane. The input frequency was swept from 1 Hz to 10 kHz.

Approximately circular arcs were obtained for DOP, DA, OAm and OA membranes as shown in Fig. 2(a); therefore these membranes may be approximated, except at a very low frequency range, by the equivalent circuit of a parallel connection of an electric resistance and an electric capacitance.

The values of equivalent resistance and equivalent capacitance were estimated from the impedance at a specified frequency where the resistance component and the reactance component of the membrane impedance were almost equal, i.e., the middle point of the

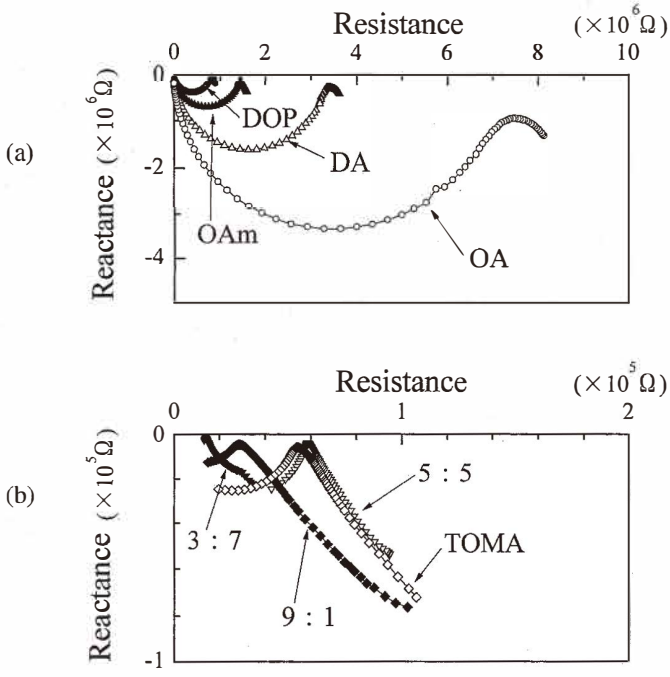

Fig. 2. Paths of the membrane impedance in a complex plane with input frequency changes from 1 to 10 kHz. (a) DOP, DA, OAm and OA membranes; (b) 9:1, 5:5, 3:7 and TOMA membranes.

circular arc. For example, the frequency was adjusted to 100 Hz for a certain measurement of the OA membrane. The resistance and the capacitance of this membrane were estimated to be 6.5 MΩ and 0.25 nF, respectively.

On the other hand, the impedances of the membranes containing TOMA, i.e., 9:1, 5:5, 3:7 and TOMA, were quite small, and the circular shapes in the paths of these membranes were limited to a narrow range as shown in Fig. 2(b). The equivalent resistance and the capacitance were therefore hard to estimate in these membranes.

3.2 *Impedance and potential change by taste substance*

The value of the membrane impedance at every measurement in the initial solution containing 10 mM KCl without added taste substances varied by ca. 30% (data not shown), whereas the membrane potential had high reproducibility. It may be inferred that the variation in the membrane impedance resulted from the difference in thickness. The membrane thickness affects the impedance, whereas the potential of the lipid/polymer membrane is theoretically independent of its thickness.[13] The ordinates of resistance and capacitance are, therefore, displayed by a relative value taken as unity as the initial value in this paper.

Figure 3 shows changes in the equivalent resistance, the equivalent capacitance and the electric potential of OA membranes with increasing concentration of the five basic taste substances. The impedance changed due to the taste substances and produced a striking feature in the equivalent circuit as follows: the resistance decreased with an increase in the concentration of taste substances as shown in Fig. 3(a), whereas no change appeared in the capacitance as shown in Fig. 3(b).

Figure 3(c) shows the dependence of the potential on the taste substances. The membrane potential increased, because the OA membranes acted as positively charged surfaces.

The membrane resistance and the membrane potential had something in common in their response to the five basic substances. Taste substances could be put in increasing order of the threshold in both the resistance shown in Fig. 3(a) and the potential shown in Fig. 3(c) as follows: quinine hydrochloride (bitterness), HCl (sourness), monosodium L-glutamate (MSG; *umami*), and NaCl (saltiness). Both the resistance and the potential showed little sensitivity to sucrose (sweetness).

Figure 4 shows the changes in the resistance and the potential of OA membranes as the concentration of two *umami* substances was increased. MSG as a kind of amino acid and disodium inosine 5'-monophosphate (IMP) as a kind of nucleotide were selected as *umami* substances in this work.

The resistance and the potential had less in common in response to MSG and IMP. The potential responses to MSG and IMP were approximately equal in both the threshold (ca. 1 mM) and slope. On the other hand, the threshold of IMP was approximately ten times lower than that of MSG in the resistance.

Thus, the responses of the resistance and the potential to taste substances had some characteristics in common as well as some differences.

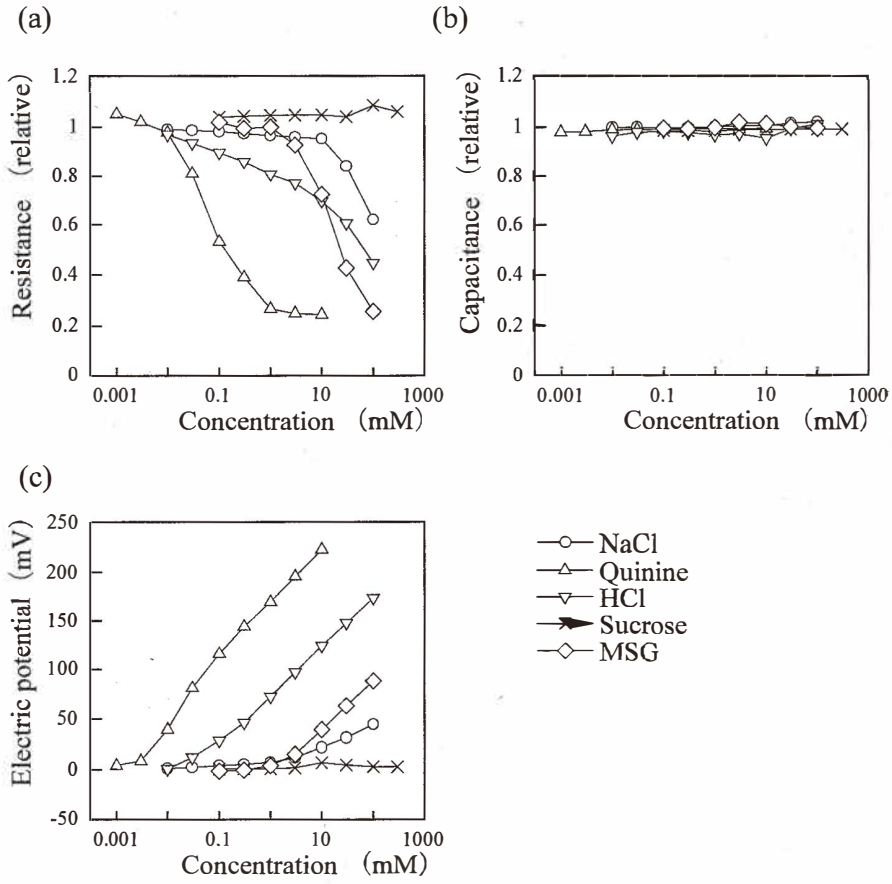

Fig. 3. Changes in electric characteristics of OA membranes due to basic taste substances: (a) the equivalent resistance, (b) the equivalent capacitance and (c) the electric potential.

3.3 *Influence of the combination of two taste substances*

The electric response to a solution of MSG and IMP in combination was measured. Figure 5 shows the changes in the equivalent resistance and the potential of OA membranes with increasing concentrations of one *umami* substance in combination with another *umami* substance in solution.

A decreasing resistance was augmented by the increasing concentration of the other *umami* substances as shown in Figs. 5(a) and 5(b). On the other hand, no remarkable effect of the combination of *umami* substances appeared in the potential as shown in Figs. 5(c) and 5(d).

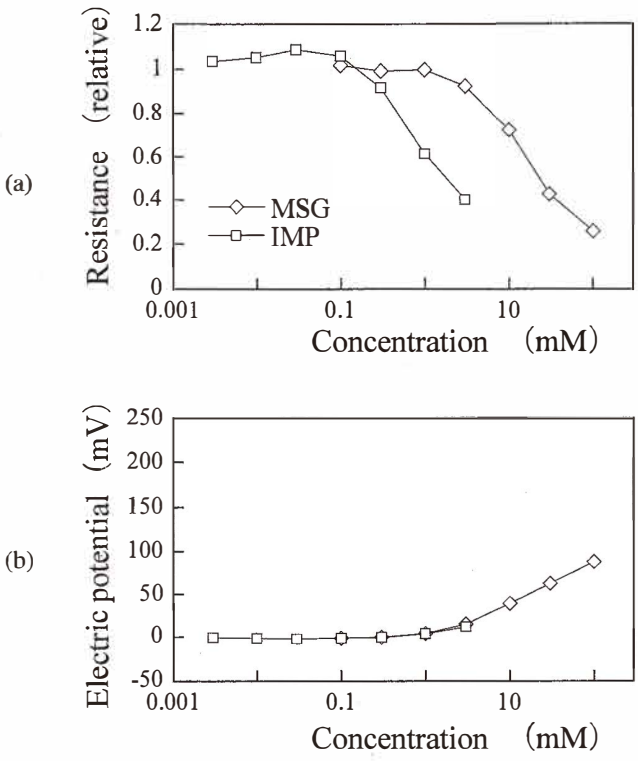

Fig. 4. Changes in electric characteristics of OA membranes due to *umami* substances: (a) the resistance and (b) the potential.

These effects of combination shown only in the resistance can be interpreted as a significant increase in sensitivity to one *umami* substance caused by adding a minute amount of another *umami* substance. This seems to be a synergistic taste effect between *umami* substances recognized empirically.

The electric responses to four basic substances in combination with a small amount of MSG were also measured. Figure 6 shows the change in the equivalent resistance and the potential of OA membranes with increasing concentrations of quinine and HCl when 3 mM MSG was added to the solution.

The responses of the resistance and the potential to quinine are shown in Figs. 6(a) and 6(c), respectively. The threshold concentration of quinine in the graph of resistance decreases well below 1 μM in combination with MSG, whereas little effect of combination with MSG appeared for the potential as shown in Fig. 6(c).

The responses of the resistance and the potential to HCl are shown in Figs. 6(b) and 6(d), respectively. In the case of the responses to HCl, the threshold concentration rose

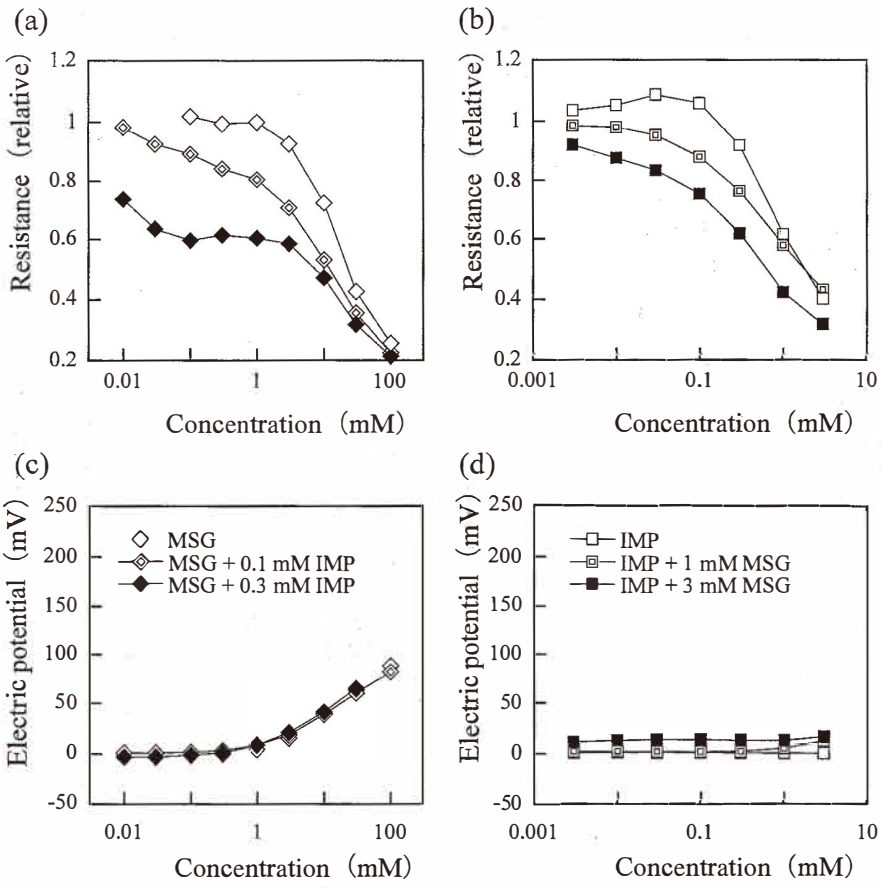

Fig. 5. Changes in electric characteristics of OA membranes due to one *umami* substance in combination with another. Resistance response to (a) MSG and (b) IMP; potential response to (c) MSG and (d) IMP.

both in the resistance and in the potential. It may be inferred that this result was influenced by the pH change in the solution when MSG neutralized the acid.

The MSG at 3 mM concentration had little effect upon all responses of the resistance and the potential to the remaining two basic taste substances, i.e., NaCl and sucrose (data not shown).

The responses of the electric characteristics of OA membranes to taste substances are shown in Figs. 3 to 6. The DOP, DA and OAm membranes also responded both in the resistance and in the potential. The responses of these membranes were different from those of the OA membrane, and the synergistic effect also appeared only in the resistance

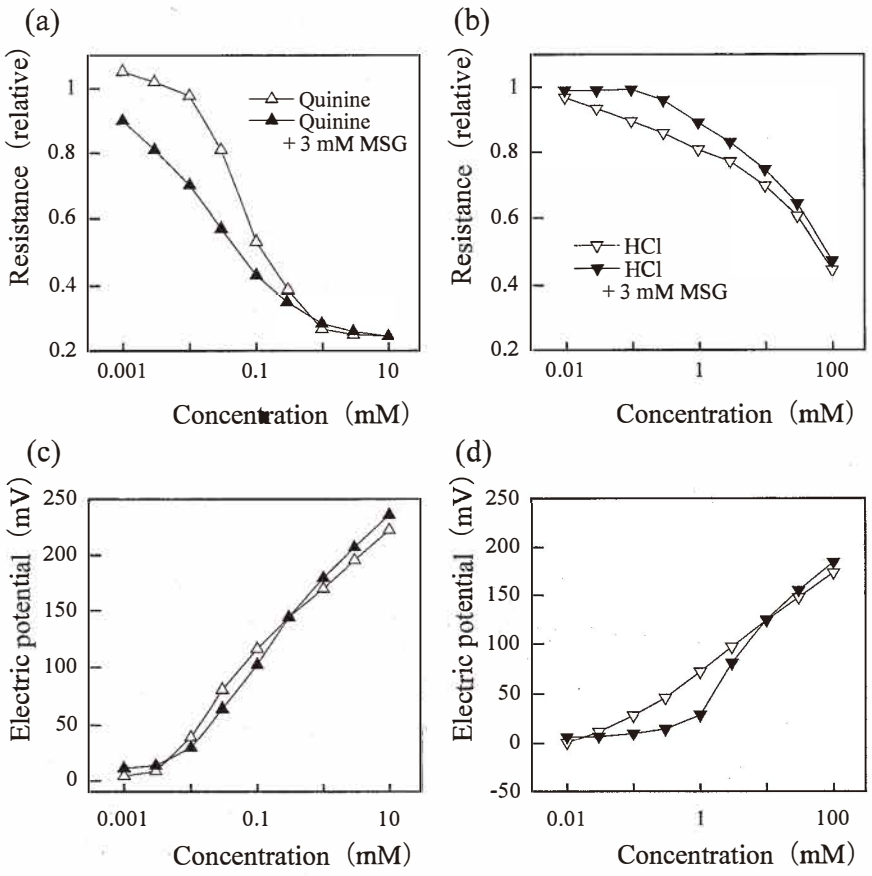

Fig. 6. Changes in electric characteristics of OA membranes due to quinine hydrochloride and HCl together with 3 mM MSG. Resistance response to (a) quinine hydrochloride and (b) HC1; potential response to (c) quinine hydrochloride and (d) HC1.

as shown in Fig. 5 (data not shown). The stability of the impedance of these membranes was, however, less than that for the OA membrane, and the reproducibility was not sufficient. The temporal stability in the impedance measurement of membranes other than the OA one must be improved.

One type of *umami* substance raised the sensitivity of the lipid/polymer-membrane impedance to the other *umami* substance as shown in Fig. 5. The MSG also raised the sensitivity to the bitter substance. It was pointed out that *umami* and bitter substances absorb into lipids.[2,12] The measurement of the membrane impedance might be affected by the strong interaction between the lipid/polymer membrane and the taste substances.

Summarizing the experimental results in this paper, a remarkable change in the membrane impedance in response to taste substances was obtained using a thin lipid/polymer membrane formed by dip-coating, and the response characteristics of the membrane impedance added useful information for the development of a taste sensor. In particular, the response difference between the impedance and the potential was notable in mixed solutions containing two taste substances. The synergistic taste effect could be detected only in the impedance measurement.

The impedance and the potential of OA membranes did not respond to sucrose. An improvement in the sensitivity to nonelectrolytes remains to be achieved. It is, however, difficult to make the characteristics of a lipid/polymer membrane completely coincide with the sensation of human taste. A measurement including the potential and the impedance of multiple membranes seems to be necessary to realize an artificial sense of taste. It is also necessary to establish an analytical method for the extraction of the information about taste from multidimensional measurements.

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