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Detection of Taste Substances Using Impedance Change in Lipid/Polymer Membranes

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The impedance changes of lipid/polymer membranes with five basic taste substances were measured. Three types of membrane consisting of different lipid molecules showed different response characteristics for membrane resistance and capacitance for taste substances. In addition, sucrose, a nonionic taste substance, was detected by a large increase in membrane resistance, whereas other taste substances induced a decrease. This result differs from those of a recently-developed taste sensor, which exhibits output changes of the electric potential of lipid/polymer membranes for electrolytes. Therefore, taste sensing based on impedance measurement uses different information from potentiometric measurements, and hence will be effective for the detection of nonelectrolyte taste substances.

1. Introduction

Taste can be attributed to physico-chemical interactions between the receptor membrane in gustatory cells and chemical substances. Electrolytes such as NaCl, HCl and monosodium glutamate (MSG) produce saltiness, sourness and "umami", respectively. Nonelectrolytes and weak electrolytes such as bitter and sweet substances also play an important role in the generation of taste from food.

Recently, biomimetic taste and odor sensing systems have been proposed by numerous researchers.[1–6] We have developed a multichannel taste sensor based on the measurement of electric potential across lipid/polymer membranes, which are interposed between a KCl

solution and the taste solution to be measured.[5,6] It responds to taste in a manner similar to a human gustatory sensation but with better reproducibility and higher resolution; i.e., the sensor output is similar for chemical substances producing similar taste qualities, whereas it is very different for those producing different kinds of taste. It was shown that the taste sensor is effective in discriminating and quantifying the taste of foodstuffs such as coffee,[7] beer,[8,9] sake[10] and milk.[11] The taste of amino acids was also quantified using the multichannel taste sensor.[12] These results may provide an objective scale of human sensory expressions.

Whereas our taste sensor based on potentiometry can detect nonelectrolytes and weak electrolytes, the sensor outputs are smaller than those for strong electrolytes such as NaCl and HCl. This is because the electric potential response in lipid membranes of the taste sensor is dependent on changes of the surface potential produced by the diffuse electric double layer,[13,14] and nonelectrolytes and weak electrolytes negligibly affect the electric double layer, even if they are bound to the membrane. In general, it is considered that physico-chemical interactions such as binding to lipids affect the structure of lipid/polymer membranes. Therefore, there is a possibility to detect such interactions by measuring changes of membrane impedance composed of electric resistance and electric capacitance. In this work, we measured the impedance of lipid/polymer membranes by the application of chemical substances which produce a taste.

2. Materials and Methods

2.1 *Lipid/polymer membranes*

The lipid/polymer membranes used in this work consisted of lipids, polyvinyl chloride (PVC) as the polymer and di-n-octylphenylphosphonate (DOPP) as the plasticizer, as already reported.[5,6] The membrane is a colorless, transparent and flexible film and its property can easily be changed by changing the lipid species mixed.[11,13] In this work, three types of lipid, dioctyl phosphate (DOP), oleyl amine (OAm) and trioctyl methyl ammonium chloride (TOMA) were used. These lipids have different head group charges, association constants of protons and states of aggregation at the oil-water interface, as demonstrated by the difference in response characteristics of these lipid/polymer membranes for taste substances.[5,6,13] A 0.01 ml aliquot from a solution of 80 mg PVC, 4 ml THF, 0.1 ml DOPP and 0.04 ml DOP (or 5 μl OAm or 0.0269 ml TOMA) was applied to coat the Ag/AgCl electrode (see also Fig.1). The width of the resulting membrane was estimated to be about 5 μm, which is one order smaller than that of the usual lipid/polymer membranes used so far.[7–12] The estimate was conducted by comparing the volume and spreading area of the solution composed of lipid, PVC, THF and DOPP on the electrode, with that of the usual lipid/polymer membrane formed in a petri dish.

2.2 *Measuring apparatus*

Figure 1 shows the experimental apparatus used to measure the impedance of the lipid/polymer membrane. The impedance of the membrane was evaluated using FRA (frequency response analyzer, NF ELECTRONIC INSTRUMENTS, 5020). The alternating

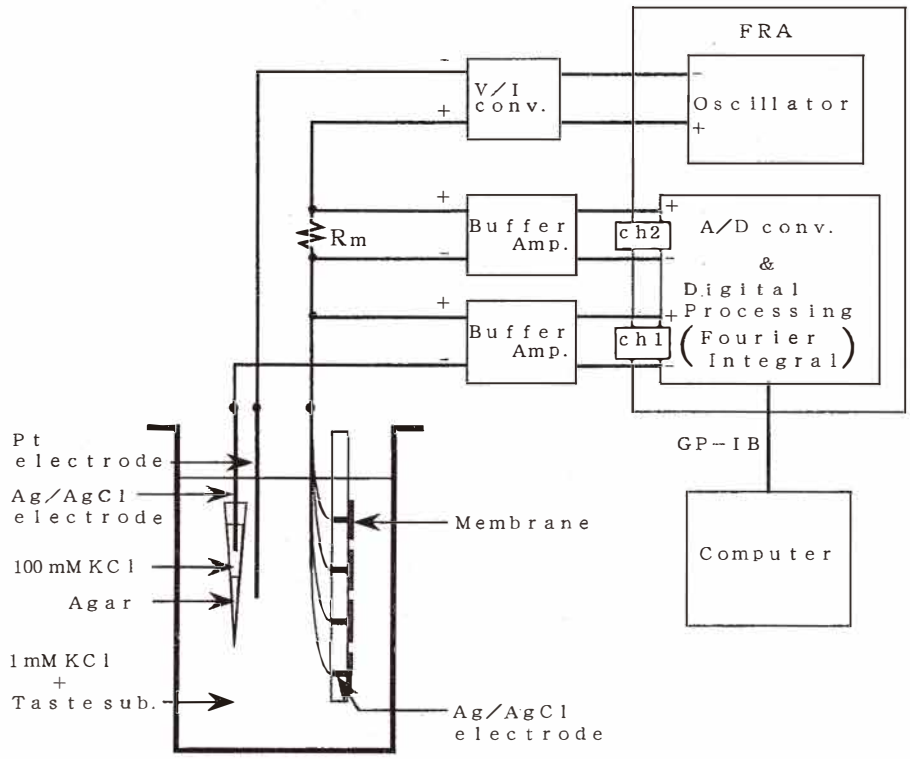

Fig. 1. Experimental apparatus to measure the impedance of lipid membrane.

input current was generated by an oscillator and a voltage/current convertor, and applied across the membrane using a pair of platinum electrodes. The input current was measured as the voltage drop across a monitor resistance (R_m) inserted into the input circuit. The output voltage across the membrane was measured using a pair of Ag/AgCl electrodes covered with agar salt. The impedance of the membrane was calculated using the ratio of amplitude and the phase difference between the input current and the output voltage.

2.3 *Taste solutions*

The substances used in this work were five chemicals that produce the basic tastes: NaCl for saltiness, HCl for sourness, quinine hydrochloride for bitterness, sucrose for sweetness and monosodium glutamate (MSG) for "umami", which is the fifth independent taste quality contained in, *e.g.*, seaweeds.[15] The taste solution used in this work was prepared by dissolving one of these chemicals in 1 mM KCl solution to the known concentration. The concentration was increased sequentially and logarithmically, and the impedance was measured at each concentration.

3. Results

3.1 *Equivalent circuit of membrane impedance*

Figures 2(a), (b) and (c) show changes in the impedances of DOP, OAm and TOMA membranes in 1 mM KCl solution, respectively, plotted on a complex plane at changing input frequencies. Half circular traces were obtained for these membranes. This result shows that the equivalent circuit of the impedance is approximately represented by the parallel-connected circuit of resistance and capacitance.

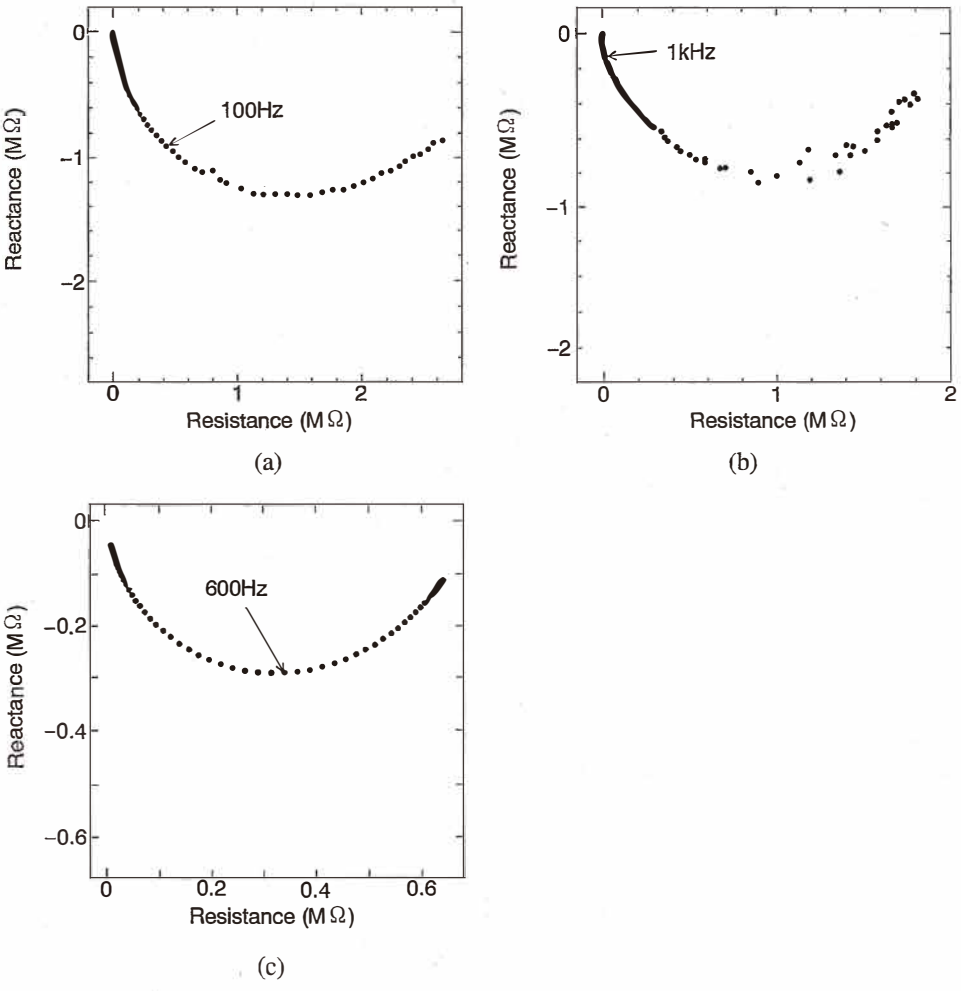

Fig. 2. Vector trace of the impedance of (a) DOP, (b) OAm and (c) TOMA membranes with input frequency change.

3.2 *Impedance changes by taste substances*

Figure 3 shows the changes of membrane resistance (ΔR) with increasing concentration of taste substance. The calculation was performed at 100, 1000 and 600 Hz input frequencies for the DOP, OAm and TOMA membranes, respectively. These frequencies

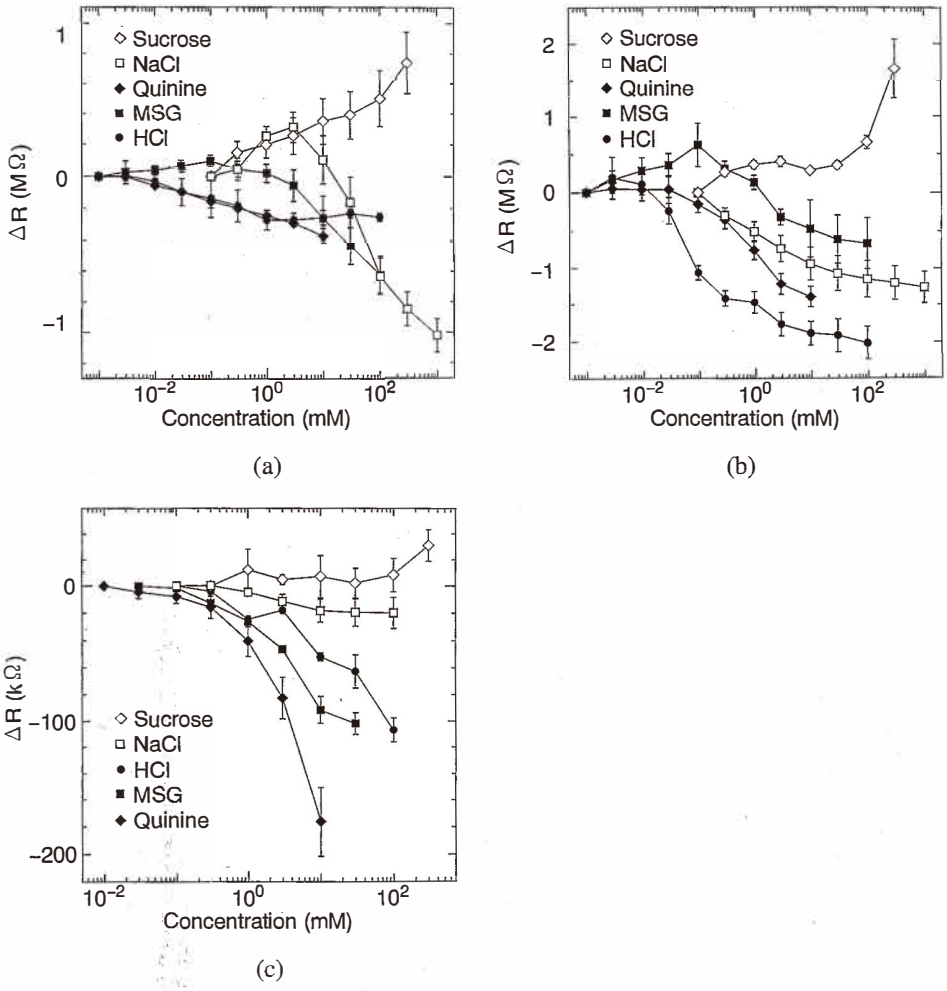

Fig. 3. Changes of the resistance of lipid/polymer membranes due to taste substances. (a) DOP, (b) OAm and (c) TOMA membranes. The averages and standard deviations shown by vertical bars were calculated using four membranes pasted on the multichannel electrode. The measurements were performed three times for DOP and OAm membranes, and five times for TOMA membranes. The same membrane was used continuously without renewal for each of measurements of five basic taste substances. All the same tendencies were obtained for these measurements.

were chosen from two viewpoints: low level of noise and relatively large magnitude of impedance on the complex plane. Whereas the membrane resistance and capacitance were calculated at different frequencies, the results scarcely changed because of half circular traces as shown in Fig. 2. The initial membrane resistance was about 1.1 MΩ in the DOP membrane and 1.8 MΩ in the OAm membrane, while it was as low as about 400 kΩ in the TOMA membrane. The resistance of the lipid/polymer membranes of DOP and OAm tended to be reduced by all of the strong electrolytes (NaCl, HCl, quinine and MSG), whereas a peak was observed for both membranes at the intermediate concentration of MSG. In a similar way, a peak appeared for NaCl response in the DOP membrane. On the other hand, sucrose (a sweet nonelectrolyte) increased the resistance of both the membranes.

The thresholds at which the changes appear are about 30 μM for HCl, quinine and MSG in the DOP membrane, while they are about 1 mM for NaCl and sucrose. In the OAm membrane, they are the same, about 30 μM for HCl and MSG, 0.1 mM for quinine, about 300 μM for sucrose and NaCl.

In the case of the TOMA membrane, the same decreasing tendency was obtained for electrolytes as HCl, MSG and quinine; however, the changes were smaller than those of the DOP and OAm membranes even if the relative changes (%) were considered in relation to the initial values. No change appeared for sucrose.

Figure 4 shows the changes of membrane capacitance (ΔC). The initial membrane capacitance was about 1.5 nF, 1.1 nF and 0.5 nF in the DOP, OAm and TOMA membranes, respectively. It increased significantly for HCl in the DOP membrane, whereas it also showed an increasing tendency for the other three substances, NaCl, quinine and MSG. However, sucrose produced no effect. In the OAm membrane, on the other hand, the relative change amounted to 10% even for MSG, which caused the largest change among five chemical substances. The changes were negligibly small for other chemical substances. In the case of the TOMA membrane, MSG caused the largest increase among electrolytes. No change appeared for the nonelectrolyte, sucrose.

4. Discussion

In a previous study[16] using lipid/polymer membranes, the changes of impedance with taste substances were very small (less than 10% of the whole value). This is because the lipid/polymer membranes had widths as large as 200 μm for obtaining stable, durable usability to measure the membrane potential using a structure of Ag/AgCl wire in inner KCl solution / membrane / reference electrode in outer taste solution. As mentioned above, however, the membranes showed only a small change of impedance with taste substances. Therefore, the membranes and the measuring structure are not suitable for measuring impedance; hence we have used a thin membrane with a lower impedance to show the large change due to taste substances by forming the membrane directly on the electrode surface.

The following discussion mainly concerns the DOP membrane, because it showed large changes in ΔR and ΔC. The thresholds, about 30 μM for HCl and 1 mM for NaCl, in the DOP membrane are of an order comparable to those obtained in measurements of

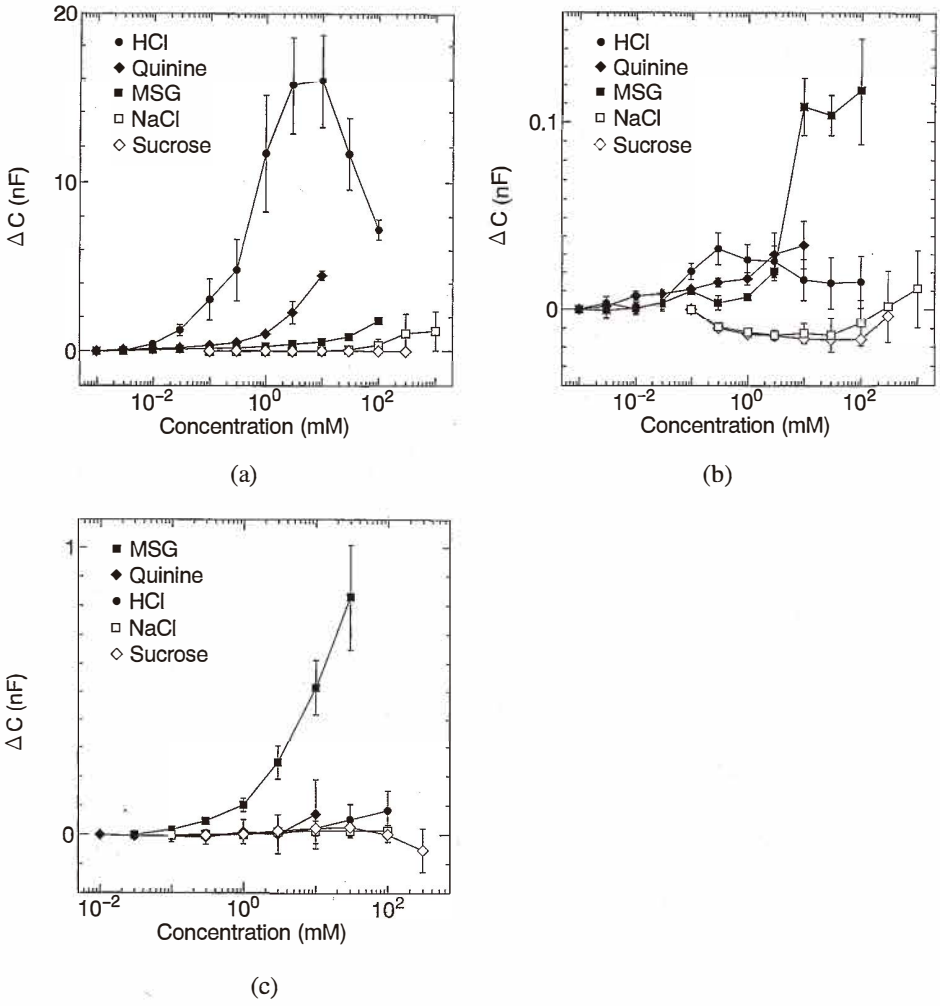

Fig. 4. Changes of the capacitance of lipid/polymer membranes due to taste substances. (a) DOP, (b) OAm and (c) TOMA membranes.

membrane potential using the conventional taste sensor[5,13,14,17] and are somewhat superior to results from human sensory evaluations.[18,19] The response to quinine shows that the threshold is higher than that obtained in the conventional taste sensor, i.e., about one μM,[13,14,17] and is comparable to that in sensory evaluations.[18,19] The threshold for MSG, about 30 μM, is lower than those of the conventional taste sensor[5] and sensory evaluations.[15] As for sucrose, the present result may be appreciable: the large increase appeared

from about 1 mM, which is much lower than about 200 mM detected by humans.[18,19]

The decreases in resistance of the membranes for NaCl, HCl, quinine and MSG in Fig. 3 can be explained as follows: molecules of strong electrolyte play the role of charge carriers within the membrane and the increase in carriers causes an equivalent decrease of resistivity of the membrane. In addition, the adsorption of quinine or MSG onto the membrane[13,14] may increase the hydrophilicity, which results in increasing permeation of ions such as K^+ and Cl^- contained in the solution.

The increase of resistance with sucrose implies that sucrose is adsorbed onto the hydrophilic surface of the membrane and may increase the packing density of the membrane equivalently or block ion permeation. It causes a decrease in ion permeation through the membrane.

These results are very similar to those obtained using cyclic voltammetry in a monolayer lipid (octadecyl mercaptan) membrane.[20] The present method seems to be far superior to the previous one from the viewpoints of ease of both membrane preparation and measurement without oxidation-reduction substances. In fact, taste itself cannot be measured, in the strictest sense, using a cyclic voltammetric measurement which requires such oxidation-reduction substances in the taste solution.

The capacitance increased significantly in the DOP membrane, which is negatively charged, with increasing HCl concentration (Fig. 4(a)). This may be due to the increase in polarization of the membrane, because the outer surface contacting the taste solution decreases its negative charge by proton binding to lipids. In fact, the increase was negligibly small in the OAm membrane for HCl (Fig. 4(b)): here Cl^- ions, which act with positively charged membranes, are scarcely adsorbed.

On the contrary, MSG increased the capacitance of the OAm and TOMA membranes. MSG is composed of negatively charged glutamate ions and Na^+ ions. Glutamate anions are shown to be bound to lipid/polymer membranes.[13] In the present case, therefore, it can be considered that the decrease in positive charge of the outer surface of the membrane resulted in increasing polarization, as in the case of the DOP membrane.

Small increases in the capacitance observed at high concentrations of NaCl in the DOP and OAm membranes may be related to the decrease in the width of the electric double layer formed near the membrane surface in an aqueous solution.

The change in resistance was small in the TOMA membrane because it has a low resistance, which implies high permeation of ions, even at low ion concentrations. Although the ion permeation may increase with increasing concentrations of electrolytic taste substances, the rate of increase is low because of its low resistance, as described below. The membrane can be regarded as a parallel connected circuit of many paths through which ions can move.[21,22] As the first approximation, we assume n ion paths in the membrane with resistance r_0. In this case, the membrane resistance R becomes r_0/n. Because the DOP and TOMA membranes have high and low membrane resistance, respectively, r_0 can be regarded as high and low. For simplicity, we assumed n does not differ greatly between these two membranes. Let us consider the simplest situation in which one path permeates ions freely with increasing concentration of electrolytes; the resistance of one path decreases to r', which is low. We can assume $r' \ll r_0$ for the DOP membrane and $r' \leq r_0$ for the TOMA membrane, because r_0 should be high and low, respectively, as above. A

straightforward calculation shows that the membrane resistance becomes $R \approx r'$ as a limiting case for the DOP membrane, whereas it changes slightly from r_0/n for the TOMA membrane. This result agrees with the observed results in Fig. 3.

It should be noted that sucrose (nonelectrolyte) could be detected as an increase of the membrane resistance of the DOP and OAm membranes. This result implies that the impedance measurement is effective for the detection of nonelectrolytes. However, the standard deviations show that the errors of repetitive measurement were large; this point must be improved so that we can apply this method to the taste sensing of common foods. If we combine two taste-sensing systems based on different principles, i.e., potentiometric and impedance measurements, a more powerful and realistic taste-sensing system can be realized.

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