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Bitterness-Masking Effect of Phospholipids on Lipid/Polymer Membranes

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Phospholipids such as phosphatidic acid exert a bitterness-masking effect without changing other taste qualities. In a previous study, we detected and qualified this suppression of bitterness using a taste sensor whose transducer was composed of several kinds of lipid/polymer membranes with different characteristics. The response to quinine hydrochloride decreased with increasing phospholipid concentration in a manner similar to human gustatory sensation. In this study, we observed the structural changes of a membrane surface using an atomic force microscope (AFM) and detected the adsorption of quinine hydrochloride and the masking effect of phospholipids. As a result of AFM measurements, we confirmed that the the masking effect was caused by blocking the adsorption of bitter substances onto to the membrane.

1. Introduction

A taste sensor with lipid/polymer membranes transforms information from taste substances into electric signals in a manner similar to the human gustatory sensation. Taste information is transformed into the pattern composed of electric signals of the membrane potentials of the receptor part. The sensor outputs are not the amounts of specific

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molecules but the taste quality, because similar patterns are obtained for substances producing the same taste quality.[1] Information on various tastes as electric signals using the sensor was successfully obtained.[2–4]

Recently, it was found that phospholipids, such as phosphatidic acid, have a bitterness-masking effect without changing other taste qualities.[5,6] It has been suggested that phospholipids bind to hydrophobic bitter substances and their receptor site on a biological membrane. In our previous study, we detected and qualified the suppression of bitterness using the taste sensor.[7] However, the mechanism has yet to be elucidated. In this study, we observed the structural changes of the membrane surface using an AFM[8] to elucidate the masking mechanism.

2. Materials and Methods

2.1 *Taste sensor*

A schematic diagram of the taste-sensing system SA402B (Intelligent Sensor Technology, Inc.) is shown in Fig. 1. The sensor was used for electrical measurements on a test solution using channels composed of eight kinds of lipid/polymer membrane.[2] The electric signals obtained from the sensor were then analyzed by principal component analysis. Decyl alcohol (DA) was used as a membrane-forming material in this study to measure bitter substances. It is a negatively charged lipid and has a high response to hydrophobic bitter substances such as quinine hydrochloride. DA (0.4 ml) was mixed with 800 mg of polyvinyl chloride (PVC) and 1 ml of plasticizer (DOPP: dioctyl phenylphosphonate) dissolved in tetrahydrofuran. The mixture was then dried on a glass plate at room temperature. The structural formula of DA is $CH_3-(CH_2)_8-CH_2-OH$. The lipid/polymer membrane is a transparent film of about 200 μm thickness.

2.2 *Measurements using taste sensor*

Figure 2 shows the structural formula of a bitter taste substance, quinine hydrochloride. In this study, we used BMI-60 (Kao Corporation) as a bitterness-masking reagent. BMI-60 is a phospholipid cocktail composed of 15–20% phosphatidic acid, 40% phosphatidylinositol, 10–15% phosphatidylethanolamine and 5% phosphatidylcholine.

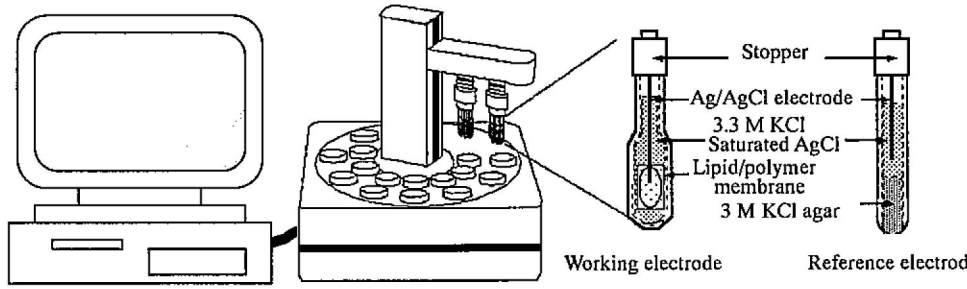

Fig. 1. Taste sensor SA402B.

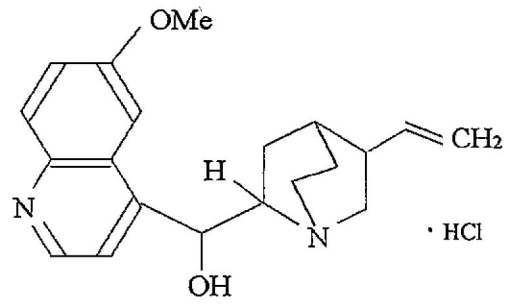

Fig. 2. Structural formula of quinine hydrochloride.

In this measurement, 0.03, 0.1, 0.3 and 1 mM quinine hydrochloride solutions were used as bitter samples; 0.5 and 1% BMI-60 solutions were used as bitterness-masking reagents. Figure 3 shows the measurement procedure. The membrane was washed in the reference solution three times for 60 s each, and the sensor measured the electric potential of the reference solution (Vr) for 15 s.

Next, the membrane was immersed in BMI-60 for 60 s, after which the sensor measured the electric potential of bitter solution (Vs). The sensor output V is the difference between the two values (Vr - Vs).

2.3 *Atomic force microscope*

A multimode SPM (Veeco Instrument) and NCH cantilever (Nanosensors™) were used in this measurement. An AFM was used to detect very small forces such as intermolecular forces and atomic forces. The static electricity of the sample was determined to strongly influence experimental results. Thus, an ionizer blowgun was used before measurements were taken because the samples in our study accumulated charge easily on their surfaces and hence produced an artifact image.

2.4 *Samples and measurements using AFM*

The compositions of the lipid/polymer membranes are the same as those described in section 2.1. The following list shows the four preconditioning procedures for each DA membrane. All membranes were cleaned using 1 mM KCl at the first and last steps. The measurements of these samples were performed using the AFM in the gas phase.

DA-1 was immersed into
1) 1 mM KCl solution for 60 s.
DA-2 was immersed into
1) 1 mM KCl solution for 60 s,
2) 1 mM quinine hydrochloride solution for 15 s,
3) 1 mM KCl solution for 3 s.

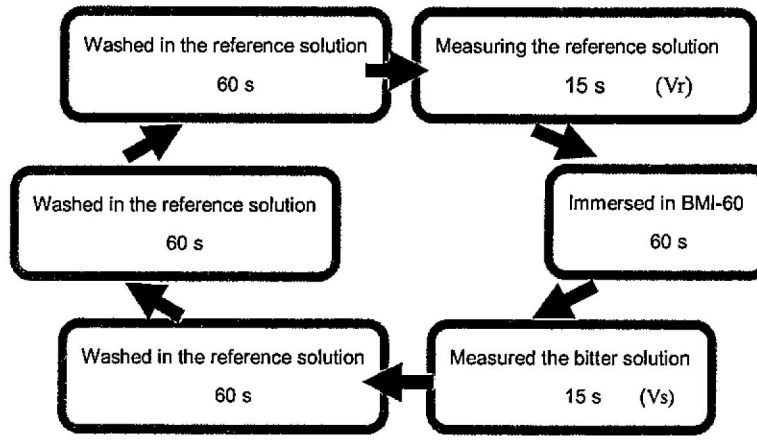

Fig. 3. Measuring procedure using the taste sensor.

DA-3 was immersed into
1) 1 mM KCl solution for 60 s,
2) 1% BMI-60 solution for 60 s,
3) 1 mM KCl solution for 3 s.

DA-4 was immersed into
1) 1 mM KCl solution for 60 s,
2) 1% BMI-60 solution for 60 s,
3) 1 mM quinine hydrochloride solution for 15 s,
4) 1 mM KCl solution for 3 s.

3. Results and Discussion

3.1 *Measurement of bitterness-masking effect using taste sensor*

Figure 4 shows the electrical response of the samples described in section 2.2. The measurement was performed three times in a rotation which involved one round of measurement for all the samples. Error bars are the standard deviations of three measurements. The responses to quinine hydrochloride without immersion in the 1% BMI-60 solution, which are the entries for 0% BMI-60 in Fig. 4, increased as BMI-60 concentration increased. The cause of such an increase on the DA membrane, which is negatively charged, was considered to be the adsorption of quinine hydrochloride, which is positively charged, onto the membrane. However, the electrical response to quinine hydrochloride after immersion in the 1% BMI-60 solution decreased with increasing BMI-60 concentration. From the results obtained here, it is roughly estimated that the electrical response to 1 mM quinine hydrochloride after immersion in the 1% BMI-60 solution corresponds to that to 0.2 mM quinine hydrochloride. Because the downward response in the DA membrane implies a decrease in the intensity of bitterness, it shows the bitterness-masking effect of BMI-60.

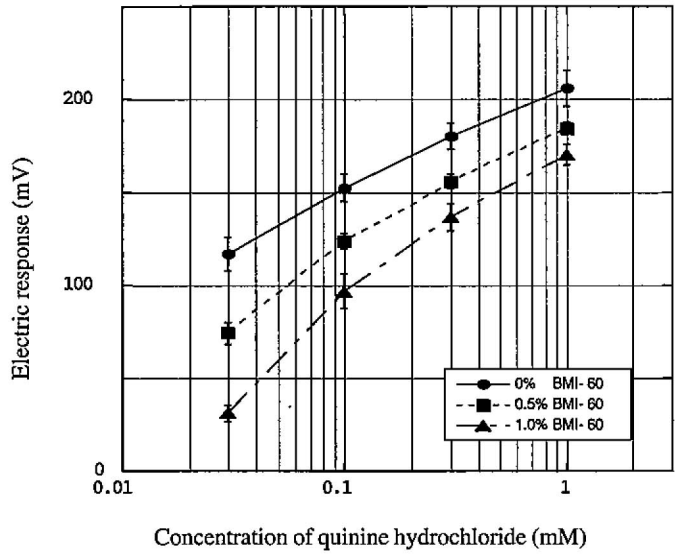

Fig. 4. Measurement of the suppression of bitterness using the taste sensor.

3.2 *Surface observations of lipid/polymer membranes using AFM*

Figure 5 shows the topographic results for the membranes listed in section 2.4 and the section analysis using AFM. Scanner drifts and image bows on the membrane surface were calibrated. As shown in Fig. 5(a), DA-1 has a very flat surface within a height of 0.5 nm. In contrast, DA-2 in Fig. 5(b), which was preconditioned by quinine hydrochloride, shows a roughness height of more than 2 nm. It is suggested that the quinine hydrochloride penetrated into the lipid/polymer membrane. DA-3 in Fig. 5(c) was preconditioned by 1% BMI-60. Its surface was relatively flat and smooth compared with the DA-2 surface; however, the surface shape was clearly different and there was a relatively large deformation compared with DA-1. It is estimated that BMI-60 was adsorbed and accumulated on the membrane surface as a result of some interaction. DA-4 in Fig. 5(d) was preconditioned by quinine hydrochloride after immersion in BMI-60. The large portion of the bitter substance and BMI-60 appeared to be removed from the membrane as compared to Figs. 5(b) and 5(c).

It is suggested that the mechanism of the bitterness-masking effect involves two events.[7] One is the direct binding of bitter substances to phospholipids, and the other is the blocking of the adsorption of bitter substances onto the membrane due to the phospholipids. Takamatsu *et al.* also discussed the mechanism of the masking effect.[9] They performed experiments under almost the same conditions as described here using HPLC and a taste sensor to quantify the amount of adsorption of quinine hydrochloride. They suggested that phospholipids were adsorbed onto the membrane surface. In such a case, BMI-60 acts as an inhibitory substance on the membrane surface and hence blocks the

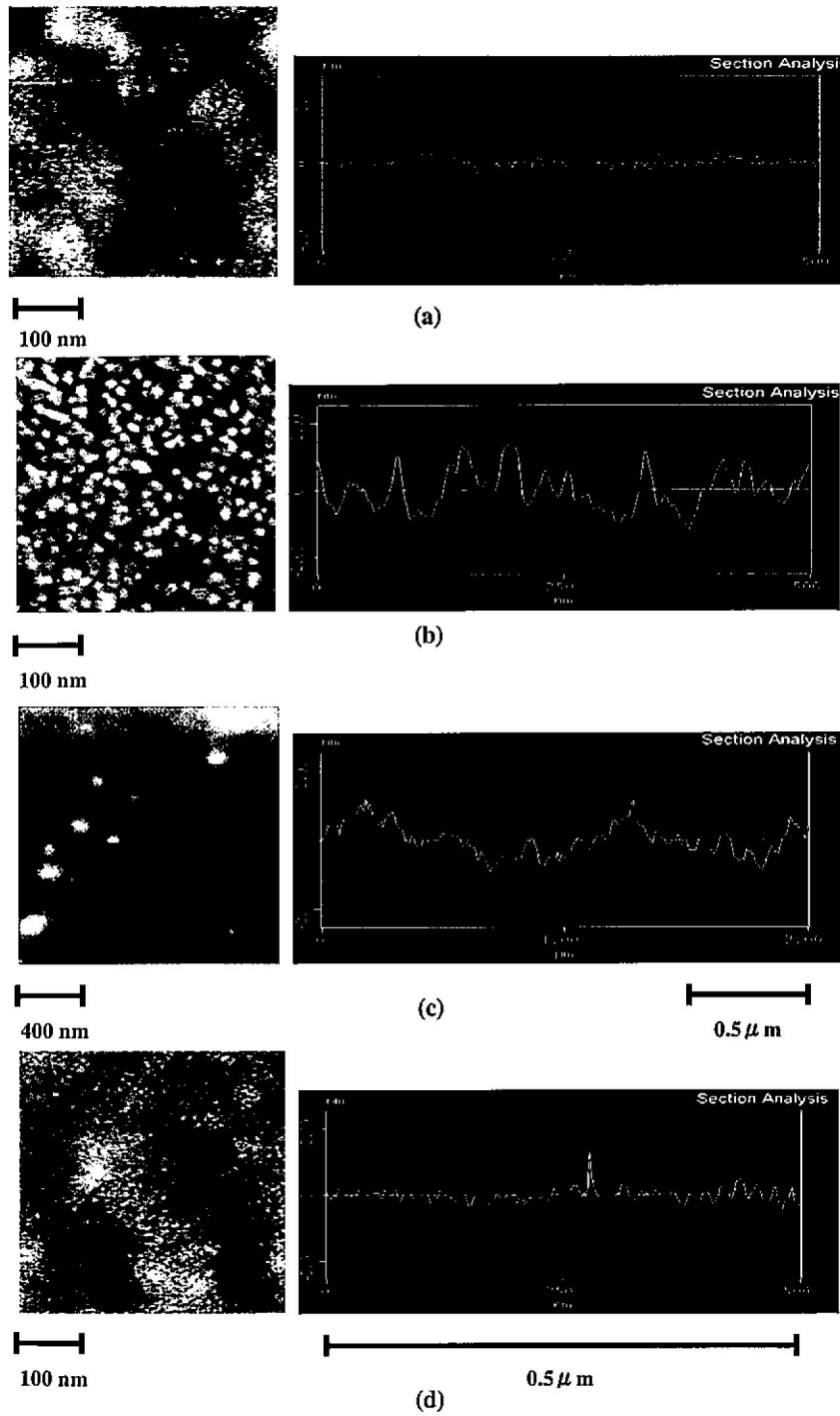

Fig. 5. Surface structures of DA membranes observed using AFM.
(a) DA-1 (400×400 nm), (b) DA-2 (400×400 nm), (c) DA-3 (1.6×1.6 μm), (d) DA-4 (400×400 nm).

adsorption of quinine hydrochloride. Our results strongly support the existence of the blocking effect of BMI-60. Phospholipids were adsorbed on the membrane surface and markedly reduced the electric potential change caused by 1 mM quinine hydrochloride down to an equivalent concentration of 0.2 mM, and the reduction was significant even at the lower concentrations of quinine hydrochloride as shown in Fig. 4.

4. Conclusion

In this study, it was shown that the taste sensor can detect the bitterness-masking effect of phospholipids. The response to quinine hydrochloride was reduced as the phospholipid concentration was increased. We observed the membrane surface using AFM and showed the adsorption of quinine hydrochloride by hydrophobic bonding and the masking effect of phospholipids. As a result of AFM measurements, it was suggested that the masking phenomenon was also caused by blocking the adsorption of bitter substances onto the membrane in addition to the direct binding of bitter substances to the phospholipids.

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