Basic Studies for the Practical Use of Bitterness Inhibitors: Selective Inhibition of Bitterness by Phospholipids

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Purpose. We examined the effects of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol (PI), and phosphatidic acid (PA) on human taste sensation to various substances.
Methods. The effects were evaluated psychophysically using paid volunteers.
Results. PA inhibited the bitterness of various substances dissolved in water without affecting sweetness, saltiness, and sourness, although its inhibitory activity was less than that of PA-LG. PI also showed inhibitory activity on bitterness, although its activity was less than PA. A soybean lecithin fraction containing high contents of PA and PI also demonstrated inhibitory activity on the bitterness of various substances. Both the incorporation of either PA or the lecithin fraction into granules containing quinine and the coating of the granules with PA or the fraction effectively inhibited the bitterness of quinine.
Conclusions. The lecithin fraction is permitted for use as an additive to drugs and food and can be produced on an industrial scale. It is expected that the lecithin fraction will be used safely as a bitterness inhibitor for practical applications.

KEY WORDS: bitter taste; taste inhibition; phosphatidic acid; phosphatidylinositol; soybean lecithin.

INTRODUCTION

Many orally-administered drugs elicit a bitter taste. In some cases, tablets and granules containing drugs with sugar or polymers have been coated (1–3) or chemically modified into insoluble derivatives (4) to mask their bitterness. However, because these methods are often insufficient in fully masking a bitter taste, a new masking method is widely required. There are also many bitter components in food, but there is no good method to mask their bitter taste.

In previous studies (5,6), we found that a lipoprotein (PA-LG) composed of phosphatidic acid (PA) and β-lactoglobulin (LG) selectively inhibits the responses of the frog taste nerve to various bitter substances without affecting responses to salts, acids, sugars or sweet amino acids. We suggested that PA-LG is bound to the hydrophobic region of gustatory cell membranes and thus masks the receptor sites for bitter substances (7). We also showed that PA-LG selectively inhibits the taste sensation of various bitter stimuli in humans (8). We prepared lipoproteins composed of different species of lipids and proteins and tested their inhibitory ability in both frogs and humans. The results showed that PA-containing lipoproteins have a strong inhibitory action, irrespective of the species of the proteins. Among the lipo proteins composed of LG and various lipids, PA-LG showed the strongest inhibitory action. For this reason, PA was considered a key material for inhibition of bitterness.

Although PA-LG is an ideal bitterness inhibitor for use in a physiological study, when actually applied to the practical purpose of inhibiting bitterness in food and drugs, it presents problems in preservation, large-scale production, and cost. In our previous studies (6,7) we noticed that PA alone inhibited the frog taste nerve response to quinine, although its inhibitory action was weaker than that of PA-LG. In the present study, we systematically examined the effects of various phospholipids, such as PA, phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE), on human bitter sensation to various substances. We also examined the inhibitory effects of a soybean lecithin fraction containing PA and PI in high content, which can be produced on an industrial scale, on the bitterness of various substances.

MATERIALS AND METHODS

Materials

The sources of bitter substances were as follows: quinine hydrochloride, quinine sulfate, promethazine hydrochloride, propranolol hydrochloride, berberine chloride, strychnine nitrate, and brucine dehydrate from the Wako Pure Chem. Ind. Ltd., Tokyo, and whey peptide and wheat peptide from DMV, Veghel. Sources of other chemicals were as follows: PE and PI from Sigma Chem Co., St. Louis; PC from Lucas Meyer, Hamburg; soybean lecithin from True lecithin Ind., Mie; corn starch from Nihon Shokuhinkako, Tokyo; hydroxypropylmethylcellulose (HPMC) from Shin-Etsu Chemical Co. Ltd, Tokyo; hydroxypropylcellulose (HPC) from Nippon Soda, Tokyo; lactose from DMV, Veghel; mannitol from Kao Corporation, Tokyo; and phospholipase D from Asahi Chemical Industry Co., Tokyo.

Methods

Preparation of Phosphatidic Acid

Phosphatidic acid (PA) was enzymatically prepared from soybean phosphatidylcholine using phospholipase D in a solvent composed of n-hexane, ethyl acetate, and 100 mM sodium acetate buffer (pH 8.0) containing 450 mM $CaCl_2$ (1:0.5:1) by slight modification of the method of Yang *et al.* (9). Purity of PA was >95% as confirmed by high performance liquid chromatography (HPLC) using a Unisil-QNH_2 column (4.6 mm × 150 mm) and elution solvent (CH_3CN/ethanol/12 mM $(NH_4)H_2PO_4$; 30/65/5).

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ABBREVIATIONS: HPC, hydroxypropylcellurose; HPMC, hydroxypropylmethylcellurose; LG, β-lactoglobulin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

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Preparation of Fractionated Lecithin

Soybean lecithin was fractionated with organic solvents. Fifty grams of soybean lecithin were washed 3 times with 500 ml of ethanol and subsequently once washed with 100 ml of ethanol/H_2O (6/4). The obtained fraction was extracted with 100 ml of n-hexane. After the removal of n-hexane with a rotary evaporator, 100 ml of cold-acetone was added to 10 g of the extract and an acetone-insoluble fraction was corrected by centrifugation. The precipitate was dried with a vacuum pump. The composition of the dried precipitate, here called fractionated lecithin, was examined by HPLC using the column and the elution solvent described above.

Preparation of Granules

Preparation of Granules Containing PA or Fractionated Lecithin. PA-containing granules were prepared by mixing quinine (1%), PA (0, 1 or 5%), lactose (54, 58 or 59%) and corn starch (40%) using microgranule manufacturing KAR-75 (Tsutsui Rika, Tokyo) and then drying the mixture. Granules ranging from 355 to 870 mm were collected with a mesh strainer. Fractionated lecithin-containing granules were prepared by a method similar to that for the PA-containing granules.

Preparation of Granules Coated with PA or Fractionated Lecithin. A mixture of quinine (1%), lactose (60%), corn starch (24%) and HPC (5%) was granulated with a Spiral flow mini (Fround Ind., Tokyo). The granules were then coated with a solution containing 5% HPMC, 0.5% polyethyleneglycol 6000, and PA of varying concentrations and then dried. Granules ranging from 355 to 1000 mm were collected with a mesh strainer. Fractionated lecithin-coating granules were prepared by a method similar to that for the PA-coating granules.

Evaluation of the Effects of Phospholipids on Human Taste Sensation

The effects of phospholipids on human taste sensation were psychophysically evaluated according to the method employed previously (10). The subjects were paid volunteers drawn mainly from staff of Kao corporation at the Kashima Food Products Research Laboratories. Between 5 and 8 subjects participated in each experiment.

The following 4 taste stimuli were used as typical substances having four primary tastes: quinine sulfate (bitter substance), sucrose (sweet substance), tartaric acid (sour substance), and NaCl (salty substance). The standard solutions of these substances were prepared by dissolving different concentrations of the substances in deionized water. Their concentrations are showen in Table 1 (11). The concentrations of standard solutions were chosen to be proportional to the intensity of their elicited taste sensations. For each, a test solution was prepared by dispersing a different concentration of a phospholipid or the fractionated lecithin using a disperser (Ultra-Turrax T25, Janke & Kunkel GmbH Co. KG, Staufenenn) in the respective solution containing the respective taste stimulus.

Evaluation of taste intensity of a test solution was carried out as follows. About 5 ml of each solution was added to a separate test tube and presented at room temperature. Subjects were required to compare the taste intensity of a test solution with that of the standard solutions and to select the standard solution having a taste intensity equivalent to that of the given test solution. Subjects compared the intensities by scooping a teaspoonful solution, placing it on the tongue, tasting it, and thoroughly rinsing their mouths with deionized water after tasting each solution.

Table 1. Concentrations of Standard Solutions Used for Psychophysical Experiments (11)

Intensity of taste	Sucrose (M) (sweet)	NaCl (M) (salty)	Tartaric acid (mM) (sour)	Quinine sulfate (mM) (bitter)
1	0.029	0.021	0.167	0.003
2	0.056	0.033	0.326	0.006
3	0.088	0.051	0.600	0.012
4	0.126	0.084	1.033	0.020
5	0.187	0.130	1.732	0.031
6	0.263	0.197	2.865	0.050
7	0.409	0.274	4.664	0.078
8	0.657	0.411	7.329	0.126
9	0.993	0.616	11.993	0.201
10	2.279	0.856	19.322	0.328

Evaluation of bitterness of granules was carried out as follows. For evaluation of the bitterness of quinine in PA- or fractionated lecithin-containing granules, the subjects put about 100 mg of the granules on the tongue and compared its bitterness intensity with that of the standard quinine solution. For evaluation of the bitterness of quinine in PA- or fractionated lecithin-coating granules, the subjects put about 100 mg of granules on the tongue and, after the granules were completely dissolved on the tongue, the subjects compared the sample's bitterness with that of standard solutions.

RESULTS AND DISCUSSION

Inhibition of the Bitterness of Various Substances in Solution

Figure 1 shows the effects of various phospholipids, such as PC, PE, PI, and PA, on sweetness of 600 mM sucrose, sourness of tartaric acid (pH 2.6), saltiness of 400 mM NaCl, and bitterness of 0.5 mM quinine. The taste intensities of sucrose and NaCl are not suppressed by any of these phospholipids. The taste intensity of tartaric acid is not suppressed by PC, PE, and PI, but suppressed slightly by a high concentration of PA. The taste intensity of quinine is not practically suppressed by PE below 0.3% but suppressed partly by 1% PE. The taste intensity of quinine is greatly suppressed by PA and PI. Thus both PA and PI are good inhibitors for bitterness of quinine.

In Fig. 2, the effects of 1.0% PA and 1.0% PI on intensities of the bitterness of various substances, including drugs and peptides are shown. Whey peptide is prepared by digesting milk proteins and elicits a bitter taste. Wheat peptide is prepared by digesting wheat proteins and also elicits a bitter taste. The values in Fig. 2 represent relative intensities of bitterness. A solution of 4.0 point elicits only very weak bitterness and that below 3.0 point elicits no sense of bitterness. Substances such as quinine, berberine, promethazine, wheat peptide, propranolol, and brucine elicited only very weak bitterness in the presence of 1% PA. The bitterness of thiamine and strychnine were moderately suppressed by 1% PA, and that of whey peptide was a little suppressed, but the suppression was not statistically

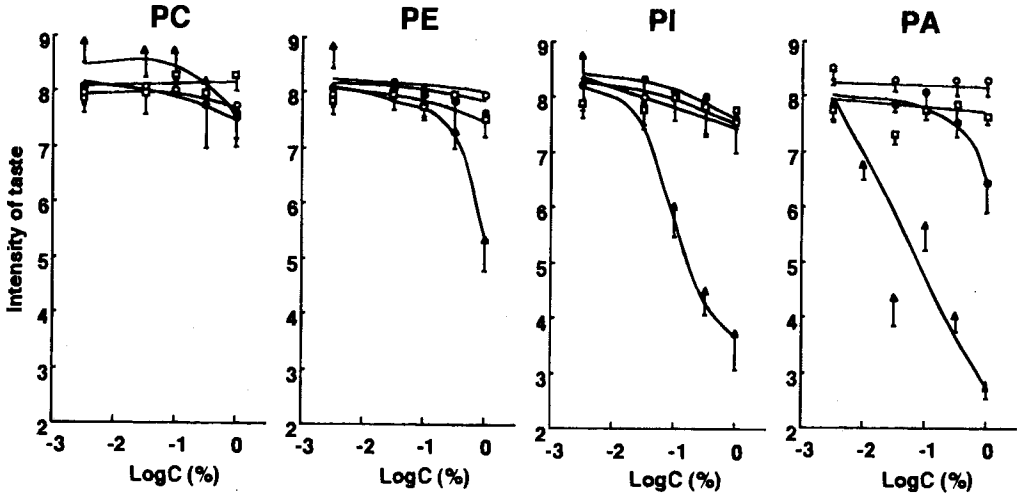

Fig. 1. Effects of various concentrations of PA on the bitterness of 0.5 mM quinine (▲), the saltiness of 400 mM NaCl (□), the sourness of pH 2.6 tartaric acid (●), and the sweetness of 600 mM sucrose (○). The intensity of taste sensation is defined in Materials and Methods. Each point in the figure represents a mean of data ± SE obtained from 5 volunteers.

significant. The bitterness of all the substances examined was sufficiently suppressed by 3% PA (data not shown). PI also suppressed the bitterness of various substances, but its suppressive effects on the substances other than berberine was much less than those of PA.

As shown above, PA greatly suppressed the bitterness of various substances. We examined how the bitterness-inhibiting effect of PA is affected by the coexistence of other phospholipids. The top column of Table 2 shows the bitterness intensity of 0.5 mM quinine solution in the presence of 0.1% PA. The addition of 0.03 and 0.1% PC did not practically affect the bitterness intensity, nor did the addition of PE. These results imply that the coexistence of PC and PE does not interfere with the inhibitory activity of PA. The addition of 0.1% PI increases the inhibitory effect on the bitterness of quinine. As shown in Fig. 2, PI itself has the inhibitory action on bitterness. Hence the increase in the inhibitory effect by the addition of 0.1% PI to 0.1% PA (control) seems to be brought about by the addition of the inhibitory effect of PI to the effect of PA. Thus, coexistence of PC, PE, and PI does not interfere with the bitterness-inhibiting effect of PA. This implies that other phospholipids do not interfere with the binding of PA to the receptor sites for bitter substances.

The results in Table 2 suggest that PA in a crude preparation containing other lipids has an inhibitory effect on bitterness. Hence we prepared a lecithin fraction containing high contents

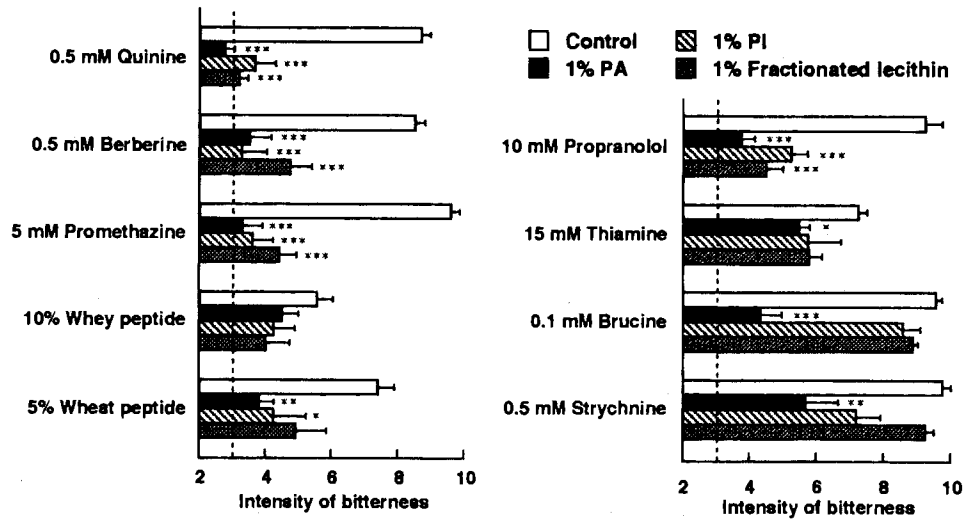

Fig. 2. Effects of PA, PI and fractionated lecithin on the bitterness of various bitter substances. The values in the figure represent the relative intensity of bitterness ± SE obtained from 5–8 volunteers. The relative intensity of bitterness is defined in Materials and Methods. A vertical dotted line at 3.0 point implies that the value below this line elicits no bitter taste. The difference between each control value and the value obtained when an inhibitor was added was evaluated by analysis of variance. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2. Effects of Addition of PC, PE, and PI to PA on Its Bitterness Inhibitory Action

Phospholipid	Intensity of bitterness
0.1% PA (control)	6.0±0.25
+0.03% PC	6.0±0.32
+0.1% PC	5.8±0.34
+0.03% PE	6.0±0.32
+0.1% PE	5.5±0.36
+0.03% PI	5.0±0.32
+0.1% PI	4.4±0.24**

Note: PC, PE, and PI (0.03 or 0.1%) were added to 0.1% PA and the effects on bitterness of 0.5 mM quinine were examined. The values in the table represent relative intensity of bitterness ± SE obtained from 5–11 volunteers. The relative intensity of bitterness is defined in Materials and Methods. Analysis of variance showed that there is statistically significant difference only between the control value and the value obtained when 0.1% PI was added (**, $p < 0.01$).

of PA and PI from soybean lecithin and examined its inhibitory effect on bitterness. Contents of PC, PE, PA, PI, neutral lipids, glycolipids, and others in fractionated lecithin were 5, 16, 18, 39, 2, 18 and 2%, respectively, while those of respective lipids in soybean lecithin were 17, 16, 5, 8, 38, 14 and 2%, respectively. The total combined percent of PA and PI in the fractionated lecithin was 57%.

Figure 3 shows the effects of the fractionated lecithin on taste sensation of 600 mM sucrose, tartaric acid (pH 2.6), 400 mM NaCl, and 0.5 mM quinine. The bitterness of quinine was greatly decreased with an increase in concentration of the fractionated lecithin, while the taste intensities of sucrose, tartaric acid, and NaCl were not suppressed even by high concentrations of the lecithin. In Fig. 2, the inhibitory effects of the fractionated lecithin on the bitterness of various substances are shown. The bitterness of quinine, promethazine, propranolol, berberine, whey peptide, and wheat peptide were decreased to below 5-point, indicating that the bitterness of these substances is greatly suppressed by the fractionated lecithin. On the other hand, the bitterness intensity of thiamine becomes 5.8, indicating that thiamine's bitterness is moderately suppressed by the fractionated lecithin. The bitterness of brucine and strychnine is only slightly suppressed by the fractionated lecithin. The bitterness of these substances was moderately suppressed by 3% fractionated lecithin (data not shown).

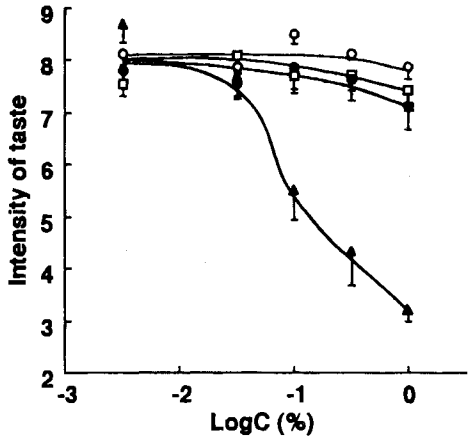

Fig. 3. Effects of fractionated lecithin on the bitterness of 0.5 mM quinine (▲), the saltiness of 400 mM NaCl (□), the sourness of pH 2.6 tartaric acid (●), and the sweetness of 600 mM sucrose (○). The intensity of taste sensation is defined in Materials and Methods. Each point in the figure represents a mean of data ± SE obtained from 5 volunteers.

Inhibition of Bitterness of Quinine in Granules

PA-containing granules were prepared by mixing 1% quinine, PA (1 or 5%), lactose and corn starch and then drying the mixture. As shown in Table 3, the addition of 1 and 5% PA to the granules decreased the bitterness point from 8.1 to 6.1 and 3.7, respectively. Thus the bitterness of quinine in the granules is practically masked by the addition of 5% PA. Note that the percentage of PA represents the weight percentage of PA to the total weight of the granules. We also examined the effect of fractionated lecithin, when incorporated into the granules, on the bitterness of quinine in the granules. Incorporation of 1 and 5% fractionated lecithin decreased the bitterness point to 6.8 and 4.3, respectively. Thus the bitterness of quinine in the granules is sufficiently suppressed by the fractionated lecithin incorporated in the granules.

We examined the effects of coating with PA on the bitterness of the granules containing 1% quinine. Practically no bitterness was sensed immediately after the granules were placed on the tongue. To evaluate the effects in more severe conditions, the intensity of bitterness was observed after the granules had completely dissolved on the tongue. As shown in Fig. 4a, the bitterness decreased with an increase in PA concentration. The data in Fig. 4a were obtained in the severe conditions, the intensity of bitterness was evaluated after the PA-coating granules were completely dissolved on the tongue, while those in Table 3 were obtained immediately after the granules were placed in the mouth. Hence it can be said that the coating of the granules with PA suppresses the bitterness of quinine much more effectively than incorporation of PA into the granules. It seems that PA in the coating layer is dispersed into saliva and effectively masks receptor sites for bitter stimuli before the drug can stimulate them. This suggests the advantages of the PA coating in that it can suppress the bitterness of a drug with a much thinner layer than can the sugar coating (10–20% sugar is usually used to coat granules) and can sup-

Table 3. Effects of PA and the Fractionated Lecithin on Bitterness of Quinine in Granules

	Intensity of bitterness
Control	8.1±0.46
1% PA	6.1±0.40**
5% PA	3.7±0.28***
1% fractionated lecithin	6.8±0.37*
5% fractionated lecithin	4.3±0.49***

Note: The granules contain 1% quinine and PA (1 or 5%) or the fractionated lecithin (1 or 5%). The values in the table represent relative intensity of bitterness ± SE obtained from 5–7 volunteers. The relative intensity of bitterness is defined in Materials and Methods. The difference between the control value and the value obtained when PA or the fractionated lecithin was added was evaluated by analysis of variance. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

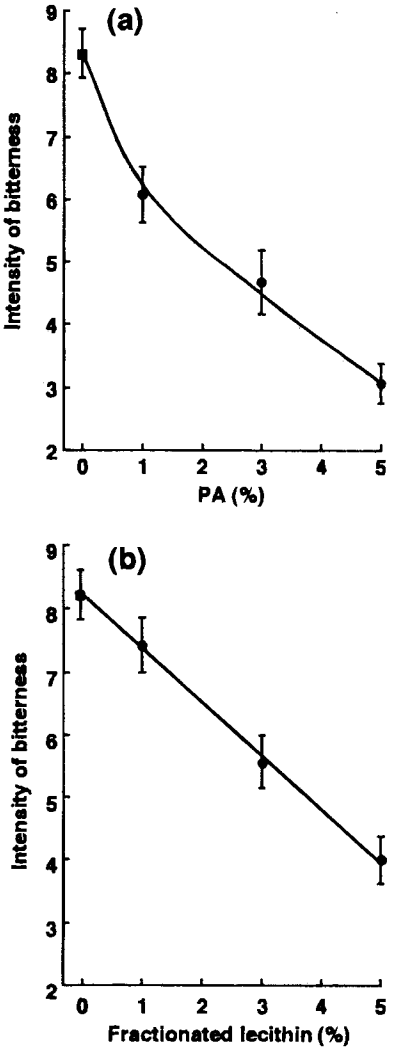

Fig. 4. Effects of coating of granules with PA (a) and the fractionated lecithin (b) on the bitterness of quinine in granules. The granules containing 1% quinine were coated with PA or the fractionated lecithin of varying concentrations. The filled square (■) represents intensity of bitterness of non-coated granules. The intensity of bitterness was evaluated after granules were completely dissolved on the tongue. Intensity of bitterness is defined in Materials and Methods. Each point in the figure is a mean of data ± SE obtained from 5 volunteers.

press the bitterness of a drug dissolved from granules in the mouth. The granules were also coated with the fractionated lecithin (Fig. 4b), and this coating also greatly suppressed the bitterness of quinine incorporated in the granules.

As described above, we examined the inhibitory effects of PA, PI and the fractionated lecithin on bitterness by three methods: addition of these inhibitors to solutions containing bitter substances; incorporation of the inhibitors to the granules containing bitter substances; and coating the granules with the inhibitors. The inhibition of bitterness was obtained most efficiently by the coating method, but the other two methods also brought about sufficient inhibition of bitterness.

A lipoprotein (PA-LG) composed of PA and LG suppressed the bitterness of all the substances examined without affecting sweet, salty, or sour tastes (5–7). In this sense, PA-LG is an ideal bitterness inhibitor for use as a physiological tool to examine receptor mechanisms of bitter substances. However, a number of problems must be overcome before PA-LG can be used practically as a bitterness inhibitor for food and drugs. LG is a protein in milk and may preclude use by those allergic to milk protein. Also, for practical applications, a bitterness inhibitor must be in good preservation in long storage and can be produceable on an industrial scale at low cost. PA-LG does not meet these demands.

In the present study, we found that PA and PI themselves had an inhibitory action on bitterness. In addition, the fractionated lecithin containing a high content of PA and PI also had an inhibitory action on bitterness. While PA-LG greatly suppressed the bitterness of all substances examined (7), PA, PI, or the fractionated lecithin did not do so. It is considered that there are multiple receptor sites for bitter substances (6). PA-LG has strong affinity to the receptors sites for bitter substances and hence mask all the receptor sites for the substances. On the other hand, PA, PI, and the fractionated lecithin have weaker affinity to the receptor sites than PA-LG, which seems to bring about variation in inhibition of bitterness.

Although the inhibitory action of the fractionated lecithin is weaker than PA-LG, there are many drugs and peptides whose bitterness was sufficiently suppressed by the fractionated lecithin. In Fig. 2, we show only the data obtained from a limited number of drugs and peptides, but the fractionated lecithin sufficiently inhibited the bitterness of many other bitter substances, including denatonium, papaverine, naringin, L-phenylalanine, and thiamine derivative, although the data are qualitative at the present. Hence, the fractionated lecithin can be used as a bitterness inhibitor for many bitter substances.

Soybean lecithin is internationally permitted as an additive to drugs and food. The fractionated lecithin belongs to a category of soybean lecithins and can be safely used as an additive to drugs and food. It is known that soybean lecithin remains stable at 40°C for 6 months in a closed dark container. The fractionated lecithin can be produced on an industrial scale at low cost. We conclude that fractionated lecithin can be used for practical purposes to inhibit the bitterness of food and drugs.

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