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ARTICLE

Surface Plasmon Resonance Analysis on Interactions of Food Components with a Taste Epithelial Cell Model

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A new device for evaluating the continuity of taste was developed with the use of surface plasmon resonance (SPR). The model of lingual cells was constructed with liposomes immobilized onto an L1 sensor chip for SPR. Using this device, we classified food components into three categories according to the sensorgram pattern and residual ratio on lipid bilayer. Samples in group A strongly interacted with lipid bilayer, those in group B poorly interacted, and those in group C belong to neither group A nor group B. Sweet proteins and gymnemic acids that prolonged sweet perception were categorized in group A. Almost all the carbohydrates investigated and aspartame, of which the taste perception does not continue, belonged to group B. This device made it possible to detect the interaction with lipid bilayer and dissected the mechanism of taste continuity.

KEYWORDS: Taste; taste continuity; surface plasmon resonance; lipid bilayer; liposome

INTRODUCTION

Food components entering the oral cavity are dissolved in saliva and come in contact with the tongue. These components are perceived by somatosensation, olfaction and gustation. The quality of foods is comprehensively evaluated by these sensations. Of these, taste is the most important factor that determines the deliciousness of foods. Tastes are divided into five basic tastes, that is sweetness, bitterness, sourness, saltiness and umami, as well as the peripheral tastes of hotness and astringency. At the same time, the characteristics of the taste are also determined by both its intensity and continuity. The continuity causes a complex sense of taste composed of aftertaste and complexity, and it contributes particularly to the better quality of tastes of foods.

To evaluate the taste, sensory tests are commonly performed, which, however, have a problem due to individual differences as well as to physical conditions of panelists. Therefore, some alternative methods should be developed for taste research, one of which is the use of molecular biology based on calcium imaging evaluating an increase in intracellular Ca^{2+} concentration in cultured cells expressing taste receptors. This event is due to the interaction of each tastant with corresponding G protein-coupled taste receptor (*1*−*3*). The increase of Ca^{2+} concentration correlates well with the taste intensity of this tastant (*4*, *5*). There is also an electrophysiological method of evaluating taste-nerve responses in taste nerve fibers, including chorda tympani, glossopharyngeal, and greater superficial petrosal nerves. The signal is converted to an electric impulse and recorded. Frogs, hamsters, mice, and rats are generally used for these analyses (*6*−*8*). Another method of analyzing taste perception involves a taste-sensor machine. This device measures a change in membrane voltage that is generated between 2 electrodes coated with lipid bilayers when the electrodes are dipped in a taste solution (*9*−*11*). Recently, a new method for analyzing the bitter-astringent substances in green tea was developed by Hayashi et al. (*12*) They used β-cyclodextrin as a sensor of these compounds conjugated with an SPR sensor chip. All these methods can analyze the intensity of taste, but no device has been available to analyze its continuity quantitatively.

This study aimed to construct a new system to analyze the interaction of food components with an epithelial lingual cell model for measuring their continuity.

The surface of the tongue is composed of epithelial cells, and the taste pores are located sparsely. We raised the hypothesis that a stronger interaction of a food component with epithelial cells results in longer taste continuity.

We used surface plasmon resonance (SPR) by which it is possible to analyze the interaction between 2 molecules in real time in complex biological processes (*13*−*15*). Because SPR can detect the dynamic interactions occurring in microflow cells, it is applicable to mimicking a food component retained in the oral cavity. We examined the interactions of various food components with the lipid bilayer.

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MATERIAL AND METHODS

Chemicals. *n*-Octyl-β-D-glucopyranoside was purchased from Sigma (St Louis, MO, USA). 1,2-Dioleoyl-*sn-glycero*-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-*sn-glycero*-3-[phospho-L-serine] (DOPS), and *N*-oleoyl-D-*erythro*-sphingosylphosphorylcholine (SM) were purchased from Avanti Polar Lipid (Alabaster, AL, USA). Glucose, galactose, fructose, mannose, lactose, sucrose, maltose, trehalose, maltotriose, raffinose pentahydrate, fucosidolactose, 1-kestose, maltotetraose, stachyose, maltopentose, glycogen from oyster, sodium carboxymethyl cellulose, arabic gum, apple pectin, citrus fruit pectin, κ-carrageenan, λ-carrageenan, xanthan gum, and locust bean gum were purchased from Wako (Osaka, Japan), galactan from Sigma-Aldrich (St. Louis MO, USA) and cellobiose from Tokyo Kasei Kogyo (Tokyo, Japan).

α-Amylase from barley malt, chicken egg white lysozyme, porcine stomach mucosa pepsin, α-chymotrypsin from bovine pancreas, type I collagenase from *Clostridium histolyticum*, tropomyosin from porcine muscle, myoglobin from equine skeletal muscle, grade VI, type II trypsin inhibitor, and riboflavin binding protein from chicken egg white (apo form), β-lactoglobulin, α-casein, α_s-casein (minimum 70%), and β-casein from bovine milk (minimum 90%), κ-casein from bovine milk, ficin from fig tree latex, and bromelain from pineapple stem were purchased from Sigma. Papain and bovine milk lactoferrin were purchased from Wako (Osaka, Japan). Aspartame, denatonium benzoate, capsaicin, and glutathione (reduced form) were purchased from Wako. Stevioside hydrate, monosodium L-glutamate (MSG), catechin hydrate, epigallocatechin gallate from green tea, and lactisole were purchased from Sigma-Aldrich (St. Louis MO, USA).

Biological Samples. Miraculin was extracted from fruits of *Richardella dulcifica* as described by Ito et al. (*16*). The cDNA of SCM was kindly provided by Kirin Company Ltd. (Tokyo, Japan). The C-terminal of the A chain of monellin was fused with the N-terminal of the B chain. A glycine residue was inserted into the fused site of the 2 subunits. The N-terminal of single chain monellin (SCM) was constructed using the third amino acid residue from the N-terminal of full-length monellin. SCM cDNA was inserted into the multiple cloning site of pET21b(+). The expression vector was introduced into *Escherichia coli* host strain BL21(DE3) pLysS for expression. The transformants were selected and cultured in LB medium for 4−5 h at 37 °C. When the absorbance at 600 nm reached 0.5−0.6, isopropyl-1-thio-3-D-galactoside (IPTG) was added to the medium at its final concentration of 1 mM. The medium was further cultured for 14−16 h. The cells were collected and lysed by sonication. The lysate was centrifuged at 7,000 rpm for 10 min at 4 °C. The separated supernatant was loaded onto a Talon metal affinity resin column, and the recombinant protein was purified. Purified SCM was dialyzed against water, and the nondiffusible fraction was lyophilized; its purity was >90% as estimated by SDS−PAGE.

Gymnemic acid was extracted from the leaves of *Gymnema sylvestre* as described by Dateo et al. (*17*). Maillard peptides were synthesized according to the method described by Katsumata et al. (*18*). Fifteen grams of soy protein isolate (Fuji Oil Company Ltd. Osaka, Japan) was dissolved in water containing 0.014 mg/mL of alkarase and incubated at 50 °C for 24 h. The alkarase was inactivated by heating at 95 °C for 10 min. The samples were kept at 4 °C and centrifuged at 5,000 rpm for 20 min. Low-molecular-weight substances were ultrafiltered using a 5,000 Da cutoff membrane. The filtrate was further ultrafiltered using a 1,000 Da cutoff membrane. Fractions ranging from 1,000 to 5,000 Da were collected and lyophilized. One gram each of these peptides was dissolved in 5 mL of water and mixed with 0.25 g of xylose. The mixture was heated at 95 °C for 4.5 h. The fraction of 1,000−5,000 Da was collected.

Analysis of Interaction with the Lipid Bilayer. Interaction analyses were carried out at 25 °C using a Biacore T100 analytical system (Biacore, Uppsala, Sweden) and an L1 sensor chip (Biacore). The L1 sensor chip consists of derivatives of a carboxymethyl dextran hydrogel and lipophilic alkanes. A lipid bilayer was formed on a sensor chip by loading liposomes.

The running buffer used for all experiments consisted of 20 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol-HCl (Tris-HCl) (pH 7.0) and 150 mM NaCl. The immobilization buffer consisted of 20 mM Tris-HCl (pH 7.0), 150 mM NaCl, and 2 mM mercaptoethanol. The washing solution contained 40 mM *n*-octyl-β-D-glucopyranoside. The L1 chip was regenerated using running buffer containing 0.25 M NaCl.

Liposome Preparation. Two types of liposome were prepared: one consisting of DOPC alone, and the other containing a mixture of DOPC, DOPE, DOPS, and SM with a weight ratio of 5:3:1:1(*19*). This ratio is from the composition of rectal gland plasma membrane. To reconstitute the phospholipids, 2 mg of each lipid was dissolved in chloroform and dried using a vacuum pump. Lipid films were hydrated in the immobilization buffer at a final concentration of 10 mg/mL. Lipid suspensions were frozen (−80 °C), thawed (20 °C), and vortexed 5 times each to ensure complete agitation. Liposomes were prepared by the extrusion method described by Mayer et al. (*20*). The lipid suspensions were extruded 25 times through a 50 nm polycarbonate filter using Mini Extruder (Avanti Polar Lipid, Alabaster, AL, USA). Liposome samples were adjusted to a concentration of 1 mg/mL and stored at −80 °C until further analysis.

Immobilization of Liposomes on the Sensor Chip. Before the addition of liposomes, the L1 chip was attached to a sensor port of Biacore T 100 system and washed with 40 mM *n*-octyl-β-D-glucopyranoside at a flow rate of 5 μL/min for 5 min. Liposomes were diluted in the immobilization buffer to a concentration of 1 mg/mL. The liposome preparations were added to the sensor chip at a flow rate of 2 μL/min for 50 min to generate a lipid bilayer on the chip. The surface of the sensor chip was washed with 0.1 M NaOH at 100 μL/min for 30 s. Thereafter, 100 μg/mL of BSA was added to the sensor chip at 5 μL/min for 5 min to cover the uncoated polydextrin with liposomes to prevent nonspecific binding. We used the below-mentioned analysis methods when the resonance units were <100.

Binding Assay. Each sample was dissolved in the sample buffer, 40 mM Tris-HCl (pH 7.0) containing 300 mM NaCl, at a concentration of 2 mg/mL and further diluted to 7 different concentrations ranging from 1 to 640 μg/mL. Insoluble samples, except for protein samples, were dissolved by heating at 60 °C. Sample solutions were injected into the microflow cell at a flow rate of 30 μL/min for 2 min, and thereafter the running buffer was eluted. Response units (RUs) were measured 3 min after the addition of samples.

Data Analysis. The SPR data were analyzed using BIAevaluation software version 4.1. The binding analysis data of samples with noise and air were omitted. Data of 5−7 concentrations were selected for statistic analysis. Response units (RUs) of the running buffer were subtracted from the RUs of the sample solution. This treatment eliminates the error caused by the order in which samples are injected.

RESULTS AND DISCUSSION

Classification of Sensorgram Patterns. Prior to examining food components, the sensorgram was classified into three categories according to the patterns (**Figure 1**). Samples in group A showed high interaction with liposome. These components were found to gradually bind to the liposome. After application of the sample, the binding components released slowly from the sensor chips. Samples in group B weakly interacted with the lipid bilayer as a rectangle-shaped sensorgram. This indicates rapid binding and fast release with no additive effects. The RUs returned to the baseline level immediately after the end of sample application. The characteristics of samples in group C differed from those of groups A and B. The components of this group exhibited weak binding to liposomes. After the end of sample application, the signals did not completely return to the baseline. A portion of the analyte formed rigid complexes with liposomes. Two possibilities are considered: that liposome is disrupted by analyte, and also that analyte binds the arm of the L1 sensor chip removed by analytes. These data were obtained with both liposomes derived from DOPC and the phospholipids mixture. The difference between DOPC liposome and mixed phospholipids liposome is little if any (data not shown). However, the reproducibility of data is higher in DOPC than in mixed phospholipids liposome, so we took the results from DOPC.

Calculation of Residual Ratio on the Lipid Bilayer. The sensorgrams showed various reaction patterns. Therefore, the same fitting program was not applied to analyze the kinetic parameters. We devised a calculation to evaluate how long food remained on the lipid bilayer. We selected 5 to 7 sensorgrams with different

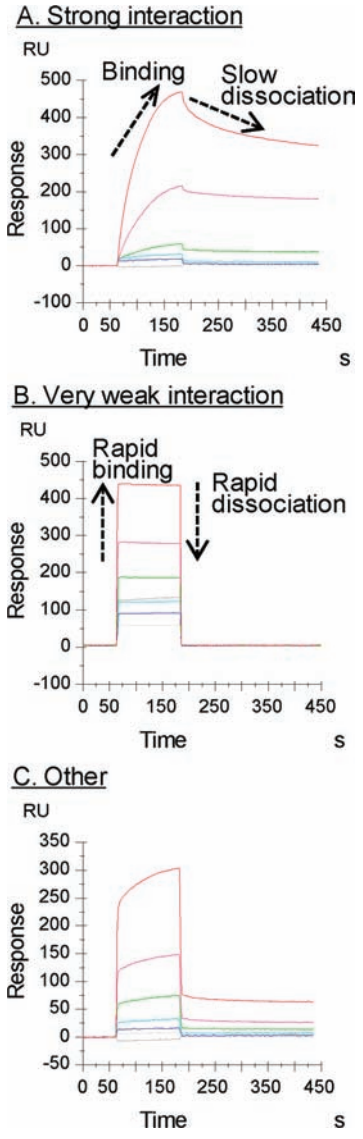


Figure 1. Classification of samples according to the pattern of the sensorgrams. (**A**) High interaction with liposomes. These components gradually bind to liposomes and release slowly. (**B**) Weak interaction with liposomes. The sensorgram is rectangle-shaped. No additive effect and fast release from liposomes. (**C**) Weak binding to liposomes. After the end of application, signals do not return completely to baseline.

concentrations and generated a putative sensorgram from the average of 5 sensorgrams. The residual ratio was calculated as follows. The RUs measured immediately before the end of sample application were divided by the RUs measured 3 min after the end of sample application (**Figure 2**). This calculation eliminates errors caused by low RUs and increases the reproducibility of the results. This method is applicable to evaluating many types of food components.

Interaction of Proteins with the Lipid Bilayer. Food proteins exhibited stronger interactions with the lipid bilayer (**Figure 3**). Several types of sensorgrams were identified (Supporting Information). Nearly all egg proteins weakly interacted with the lipid bilayer. Among milk proteins, the interaction ratio of lactoferrin was high and caseins, which form micelles in milk, strongly interacted with the lipid bilayer. In contrast, β-lactoglobulin weakly interacted with the lipid bilayer. Cysteine proteinases (CPs) present in plants such as bromelain and papain strongly interacted with the lipid bilayer. Even though ficin is a CP, it exhibited a weak interaction with the lipid bilayer, and its residual ratio on the lipid bilayer was low. At high concentrations, i.e. 160 μL/mL (data not shown), the lipid bilayer detached from the sensor chip. This reason for strong interaction of CP with lipid bilayer is unknown. Thaumatin, single chain monellin (SCM), and miraculin strongly interacted with the lipid bilayer. These proteins contribute to the continuity of taste. Both monellin and thaumatin are potent sweeteners sensed by the sweet taste receptor T1R (*21*, *22*). Thaumatin is received by T1R2 and monellin by the amino-terminal domain of TIR2 (*23*, *24*). A potential mechanism of continuity is that, although sweet taste perception is generated after being sensed by the receptor, the taste continuity must occur in the interaction with epithelial cells. The molecules bind to lingual epithelium and dissociate gradually, thus reaching the taste pores slowly to lengthen the continuity of taste. As a result, the sweetness of thaumatin and SCM persisted for several minutes. Miraculin is a taste-modifying protein that converts sourness to sweetness, and its activity persists for one hour (*25*). Strong interaction with the lipid bilayer should lead to continuity. These compounds have strong interaction with liposomes. There are papers reported to have high interaction with lipid layer under physiological conditions. Lactoferricin is generated by the pepsin-mediated digestion of lactoferrin. Lactoferricin interacts to phospholipid monolayers and bilayers (*26*, *27*). Therefore, lactoferrin may show a strong interaction with lipid bilayer. Peanut lectin has been associated with human oral epithelial cells *in vivo* (*28*). Soybean agglutinin (SBA) binds to the surface of the cell of corneal endothelium, disrupting its monolayer (*29*). This event showed that SBA interacts with the surface of the cell, and does not contradict our data showing that SBA strongly interacts with liposome.

Interactions of Carbohydrates with the Lipid Bilayer. We analyzed the interaction between lipid bilayers and various carbohydrates contained in foods via SPR. Monosaccharides, disaccharides, triose, tetrose, and pentose exhibited a box-shaped sensorgram and their residual ratios were close to nie (**Figure 4**). Thus, these sugars no additive effect and fast release with the lipid bilayer (Supporting Information). κ-Carrageenan, apple pectin, arabic gum, and xanthan gum showed rectangle-shaped sensorgrams, but their RU values did not return to baseline. Therefore, their residual ratios were around 0.1 (**Figure 4**).

Certain polysaccharide solutions are viscous and are widely used as thickeners. In the present study, these thickeners did not show interaction with the lipid bilayer, thereby suggesting that polysaccharide thickeners reduce the diffusion speed of soluble tastants. Indeed, taste intensity decreases when tastants are dissolved in viscous polysaccharide solutions. Thus, the viscosity of polysaccharide solutions enhances the continuity of taste and reduces the intensity.

Interaction of Some Taste Substances. We further investigated the interaction between low molecular weight tastants, such as aspartame, stevioside, denatonium benzoate, capsaicin, and catechins, and the lipid bilayer (**Figure 5** and Supporting Information). A strong interaction with the lipid bilayer was observed only in catechins. The residual ratio of EGCG to the lipid bilayer was higher than that of other catechins. In terms of the sensorgram pattern, EGCG and catechin are similar to each other, but the residual ratio of EGCG was higher than that of catechin. The interaction of catechins with the lipid layer was also analyzed by quartz crystal microbalance (QCM) (*30*) and molecular dynamics (*31*). From the results, it is inferred that EGCG has the strongest interaction with the lipid bilayer. The strong interaction of EGCG was caused by a gallate moiety that forms hydrogen bonds with hydrophilic sites of liposome (*32*−*34*). Our results coincide with these previous examinations.

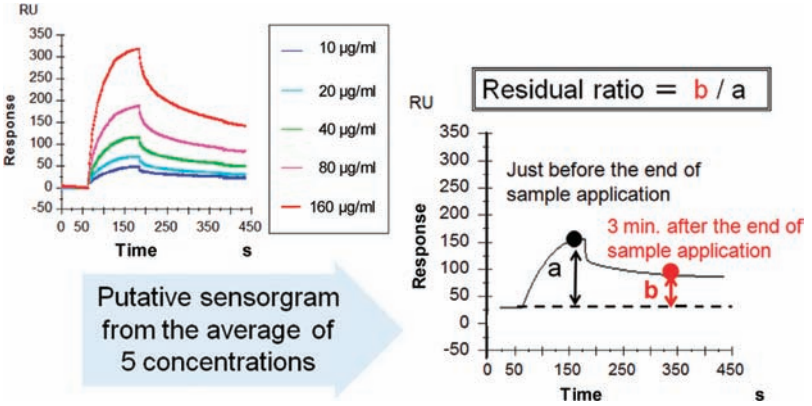


Figure 2. Calculation of residual ratio on lipid bilayer. Five sensorgrams of different concentrations were selected, and the average sensorgram was determined. The residual ratio was calculated as RUs measured just before the end of sample application (*a*)/RUs measured 3 min after the end of sample application (*b*).

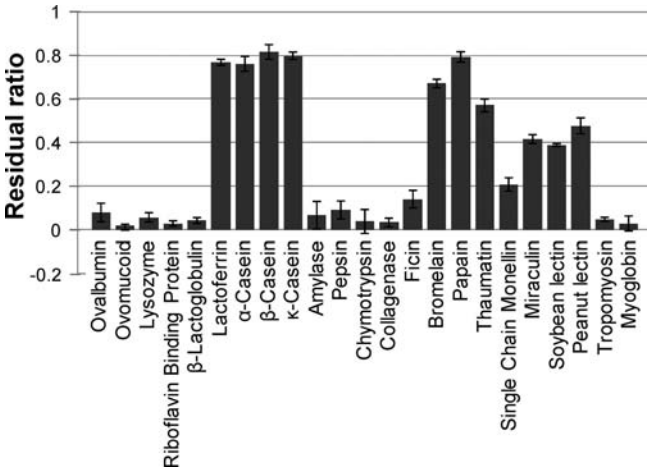


Figure 3. Residual ratios of food proteins to the lipid bilayer constructed with DOPC liposome. Each bar represents the standard error (n = 3).

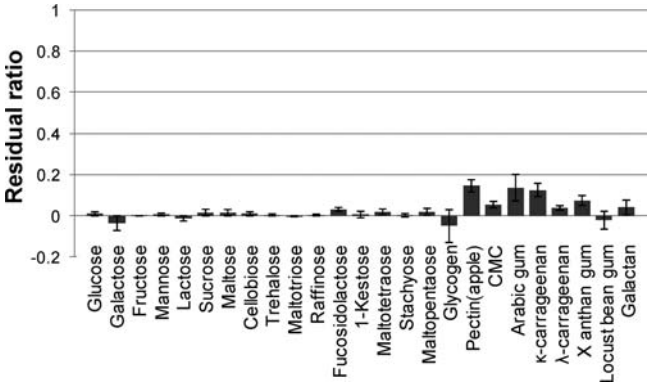
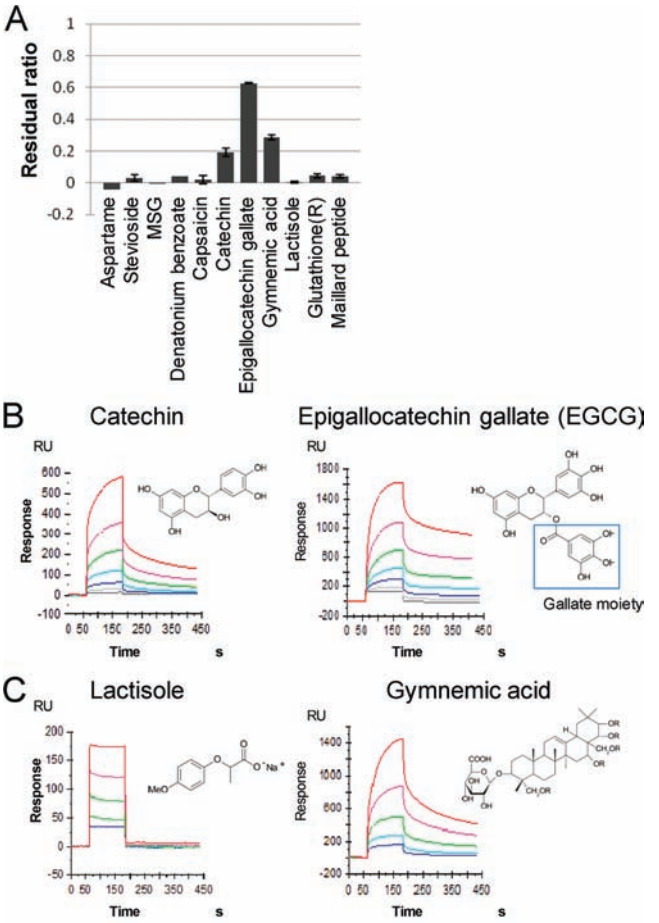


Figure 4. Residual ratios of carbohydrates to a liposome constructed with DOPC. Each bar represents the standard error (n = 3). CMC shows carboxymethyl cellulose.

Next, we examined certain taste-modifying molecules that influence taste sensation. Glutathione and Maillard peptides are reported to possess a "kokumi" taste element that prolongs taste and enhances umami (*18*, *32*, *35*). These components both weakly interacted with the lipid bilayer.

Mechanism of Development of the Kokumi Taste. The word "kokumi" refers to continuity of complexity and spread of taste, and in particular, the umami taste (*36*). In addition, kokumi taste



	Sweet suppressive activity	Continuity	Interaction with lipid bilayer
Gymnemic acid	Strong	Strong	Strong
Lactisole	Strong	Weak	Weak

Figure 5. Interaction of tastants with lipid bilayer. (**A**) Residual ratios of some tastants to DOPC. Sweeteners, umami substance, bitter substance, hot substance, sweetness suppressors, and kokumi substance. Each bar represents the standard error (n = 3). Glutathione(R) shows reduced form of glutathione and MSG, monosodium glutamate. (**B**) Structures and sensorgrams of catechins. (**C**) Structures and sensorgrams of sweet taste suppressors. R in gymnemic acid structure means H or acyl residues. The concentrations of each sample are shown in the Supporting Information.

refers to the enhancement of the continuity of taste. In the present study, glutathione and Maillard peptides did not interact with the lipid bilayer; therefore, in the physiological setting, these kokumi substances may prolong taste perception by interacting with tastants and not with the epithelial cells of the tongue. The kokumi tastant, glutathione, is reported to be received by calcium-sensing receptor (*37*). This reaction with receptor may also induce the taste continuity. MSG did not interact with liposome in our study. However, the long-lasting taste of MSG is well-known. The long-lasting taste of MSG may be induced by some other mechanisms.

Both lactisole and gymnemic acid are well-known sweet taste suppressors. The activity of gymnemic acid persisted and strongly interacted with the lipid bilayer, whereas lactisole activity did not persist and its interaction with the lipid bilayer was poor.

These findings suggest that stronger interaction with the lipid bilayer generates longer continuity in terms of sweet taste suppressive activity. Gymnemic acid is a strong suppressor of sweetness (*17*). The mechanism of action was investigated in cultured cells expressing the human sweet taste receptor. Another sweetness suppressor, lactisole, was also found to interact with the transmembrane region of T1R3 (*38*). These sweetness suppressors differ in terms of their effects on the continuity of taste. The effect of lactisole does not exceed 30 s, whereas that of gymnemic acid continues for >1 h (*17*). In the present study, only gymnemic acid strongly interacted with the lipid bilayer and was retained there. These findings indicate that the prolonged sweetness suppressive activity of gymnemic acid arises from its strong interaction with the lipid layer. In the physiological setting, gymnemic acid may be retained on the epithelial cells of the tongue and may be gradually dissolved by saliva; thereafter, it may gradually enter the pores of the taste buds and inhibit the binding of sweeteners to T1R. Another possibility of taste receptor or downstream components of its signaling system would be raised.

We constructed a device to detect the interaction of model lingual epithelial cells and dissected partially the mechanism of taste continuity. Though other parameters including the effect of saliva or the activation of receptor system may also concern taste continuity, our approach is useful for analyzing taste continuity from the viewpoints of interaction with the lipid layer.

ABBREVIATIONS USED

SPR, surface plasmon resonances; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-*sn-glycero*-3-[phospho-L-serine]; SM, *N*-oleoyl-D-*erythro*-sphingosylphosphorylcholine; SCM, single chain monelin; CP, cysteine proteinase; EGCG, epigallocatechin gallate; CMC, carboxymethyl cellulose; QCM, quartz crystal microbalance; Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol.

Supporting Information Available: Figure depicting the sensorgrams of various food components. This material is available free of charge via the Internet at http://pubs.acs.org.

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