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Establishment of a New Cell-Based Assay To Measure the Activity of Sweeteners in Fluorescent Food Extracts

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Supporting Information

ABSTRACT: Taste receptors have been defined at the molecular level in the past decade, and cell-based assays have been developed using cultured cells heterologously expressing these receptors. The most popular approach to detecting the cellular response to a tastant is to measure changes in intracellular Ca^{2+} concentration using Ca^{2+}-sensitive fluorescent dyes. However, this method cannot be applied to food-derived samples that contain fluorescent substances. To establish an assay system that would be applicable to fluorescent samples, we tested the use of Ca^{2+}-sensitive photoproteins, such as aequorin and mitochondrial clytin-II, as Ca^{2+} indicators in a human sweet taste receptor assay. Using these systems, we successfully detected receptor activation in response to sweetener, even when fluorescent compounds coexisted. This luminescence-based assay will be a powerful tool to objectively evaluate the sweetness of food-derived samples even at an industry level.

KEYWORDS: Cell-based assay, luminescence, fluorescence, aequorin, clytin-II, photoprotein, taste, sweet taste receptor

INTRODUCTION

Over the past decade, mammalian taste receptors that play a major role in tastant recognition have been found.[1] The perception of sweet, umami, and bitter tastants is mediated by G protein-coupled receptors (GPCRs): that is, T1R2/T1R3 for sweet,[2] T1R1/T1R3 for umami,[3] and T2Rs for bitter tastants.[4,5] In vitro assay systems using cultured mammalian cells heterologously expressing each taste receptor together with a chimeric G protein, for example, G16gust44 and G15i3, have been investigated not only to characterize the existing ligands for novel taste receptors but also to screen new taste modulators.[6,7] After loading taste receptor-expressing cells with Ca^{2+}-sensitive fluorescent dyes, for example, fluo-3, fluo-4, and fura-2, cellular responses can be easily detected by measuring the changes in the fluorescent intensity produced by those indicators. Although fluorescent indicators are known to be suitable for evaluating various types of natural and artificial compounds, problems remain. In particular, in the food research field, samples containing food-derived compounds or fractions are difficult to evaluate because food often contains a variety of fluorescent substances. Some are endogenous in food materials, such as polyphenols in plants,[8] and others are generated during heat processing or fermentation.[9] In considering the industrial importance of taste-modulating compounds of food origin, establishing a high-throughput screening system that can be applied to evaluate fluorescent food-derived materials will no doubt be necessary.

There are techniques available for measuring the intracellular Ca^{2+} concentrations of cultured cells without using fluorescent Ca^{2+} indicators. One method is to utilize jellyfish-derived Ca^{2+}-sensitive photoproteins, such as aequorin,[10,11] obelin,[12−14] and clytin,[15,16] as calcium indicators. The active photoprotein is composed of apophotoprotein and luciferin coelenterazine in the presence of molecular oxygen.[17,18] Binding of Ca^{2+} to the holoprotein induces a conformational change in this protein and triggers the oxidation of coelenterazine to coelenteramide. Because these events induce the subsequent emission of blue light, measuring emission of this light reveals the response mediated by changes in Ca^{2+} concentration without the use of a fluorescent Ca^{2+} indicator. Many groups have reported cell-based assay systems that use photoproteins as detectors for several types of GPCRs[19−22] and ion channels.[23] Because of the essentially undetectable background luminescence of cells, luminescence-based assays provide a high signal-to-noise ratio. Moreover, the localization of photoproteins to mitochondria results in stronger signals in GPCR assays because the rise in Ca^{2+} concentration upon IP_3-induced Ca^{2+} mobilization from intracellular stores is more pronounced in the mitochondria than in the cytoplasm.[24,25]

Although many photoprotein-based functional assays for various types of receptors have been reported, this system has not yet been adapted to analyze taste receptors. To apply a luminescence-based assay for taste receptor evaluation, in this study, four types of photoproteins were transiently transfected into cells that stably coexpressed the human sweet taste receptor, hT1R2/hT1R3, together with a functional chimeric G-protein, hGα16gust44,[26] and the cellular responses to sweetener were measured using a luminescence microplate reader. By comparing the luminescence intensities and signal/background ratios obtained using each photoprotein with or without mitochondrial localization, we selected the most suitable apophotoprotein and coelenterazine analogue for the taste receptor assay. This luminescence-based assay platform enables us to perform an otherwise impossible assay of evaluating the sweet taste intensity of samples per se containing fluorescent substances.

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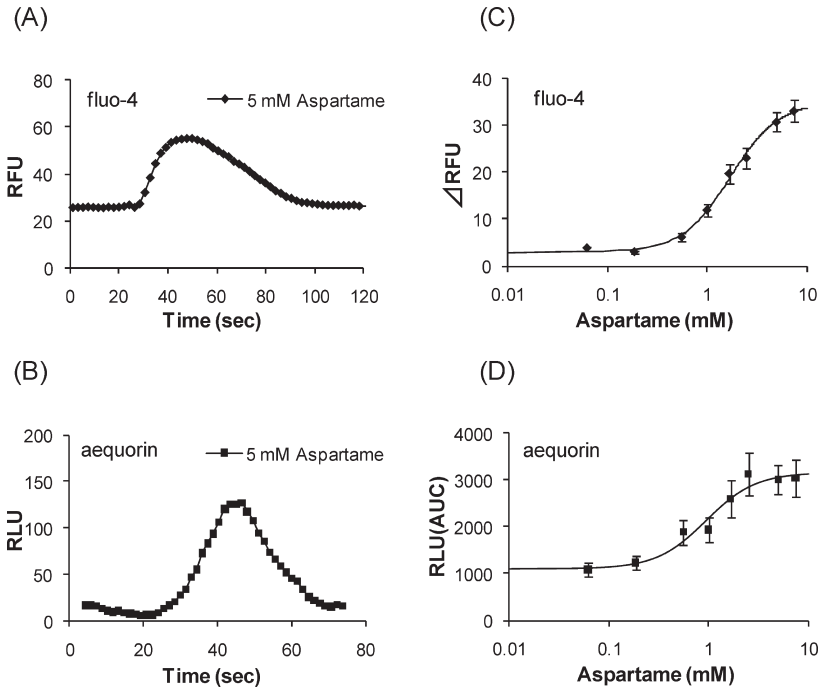

Figure 1. Comparison of the signals obtained using the luminescence assay vs the fluorescence assay. (A) The fluorescence intensities obtained using the fluo-4 assay when the hT1R2/hT1R3-expressing cells were stimulated with 5 mM aspartame ($n = 6$). (B) The luminescence intensities obtained using the aequorin assay when the hT1R2/hT1R3-expressing cells were stimulated with 5 mM aspartame ($n = 6$). (C and D) Dose−response curve of hT1R2/hT1R3-expressing cells to aspartame using the fluo-4 assay (C) or the aequorin assay (D). Each point on the dose−response curves indicates the mean ± SEM ($n = 6$).

MATERIALS AND METHODS

Materials. Samples were obtained from commercial sources: Aspartame and riboflavin were purchased from Wako Chemical Co. (Osaka, Japan), BCECF [2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein] was from Invitrogen (Carlsbad, CA), coelenterazine was from Promega (Madison, WI), and its derivatives were from Sigma Aldrich (St. Louis, MO). The buffer for the fluorescence assay was composed of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, and 1.2 mM $MgCl_2$ (pH adjusted to 7.4 using NaOH). The assay buffer for the luminescence assay was composed of the fluorescence assay buffer with the addition of 0.1% bovine serum albumin (BSA, Sigma Aldrich). The ligands were diluted into each of the assay buffers at the desired concentrations.

Construction of the Apophotoprotein Expression Plasmids. The nucleotide sequences of the cDNA fragments encoding the apophotoproteins used were codon-optimized for expression in mammalian cells using OptimumGene (GenScript), and the optimized cDNAs were chemically synthesized by GenScript Inc. (Piscataway, NJ). The Kozak-consensus sequence was introduced upstream of the start codon for efficient translation. The apophotoproteins were targeted to mitochondria by fusing the nucleotide sequence encoding the first 29 amino acids of the N-terminal leader sequence of cytochrome *c* oxidase (subunit VIII) immediately before the ATG triplet coding for the initiator Met in each apophotoprotein-coding sequence. The constructs containing the apophotoprotein-coding sequences were subcloned into the *Asc* I-*Not* I site of the pEAK10 expression vector (Edge Biosystems, Gaithersburg, MD).

Culture of Cells Expressing the Human Sweet Taste Receptor. Flp-In 293 cells stably expressing the human sweet taste receptor, hT1R2/hT1R3, together with the chimeric G-protein, hGα16-gust44, were constructed as previously reported.[26] The cells were maintained in low glucose concentration (1.0 g/L) Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% fetal bovine serum (Invitrogen) at 37 °C under 5% CO_2.

Fluorescence Assay. The cells were seeded at a density of 90000 cells/well into 96-well black-walled CellBIND surface plates (Corning, NY) and cultured at 37 °C under 5% CO_2. After a 24 h of incubation, the cells were washed with assay buffer, loaded with 5 μM fluo-4AM (Dojindo Laboratories, Kumamoto, Japan) in assay buffer for 30 min at 27 °C in the dark, and washed again with assay buffer. Changes in fluorescence intensity (excitation at 485 nm, emission at 525 nm, and cutoff at 515 nm) were monitored using a FlexStation 3 microplate reader (Molecular Devices Co., Sunnyvale, CA) at 2 s intervals. After 20 s of baseline reading, an aliquot of assay buffer supplemented with 2× ligand was added, and the scanning was continued for an additional 100 s. The response was expressed as the ΔRFU (delta relative fluorescence units) calculated as the difference between the maximum and the minimum fluorescence values. The responses were averaged from at least three wells receiving the same stimulus. The signal/background ratios and EC_{50} values for the ligand−receptor interactions were determined from the concentration−response curves generated using Clampfit ver. 9.2.0.09 (Molecular Devices) by fitting the data to the Hill's equation. The signal/background ratio was determined as the maximum signal/minimum signal.

Luminescence Assay. Human sweet taste receptor-expressing cells were seeded in six-well culture plates and transfected with 4 μg of the apophotoprotein expression plasmid using Lipofectamine 2000 regent (Invitrogen). After 6 h, the transfected cells were trypsinized, seeded in 96-well black-walled CellBIND surface plates at a density of 100000 cells/well, and cultured overnight at 37 °C in 5% CO_2. After overnight culture, the medium was removed and replaced with coelenterazine loading buffer (luminescence assay buffer containing 10 μM coelenterazine, pH 7.4) for 4 h at 27 °C in the dark. After 20 s of baseline reading, an aliquot of the luminescence assay buffer supplemented with 2× ligand was added, and the light emission was recorded using a

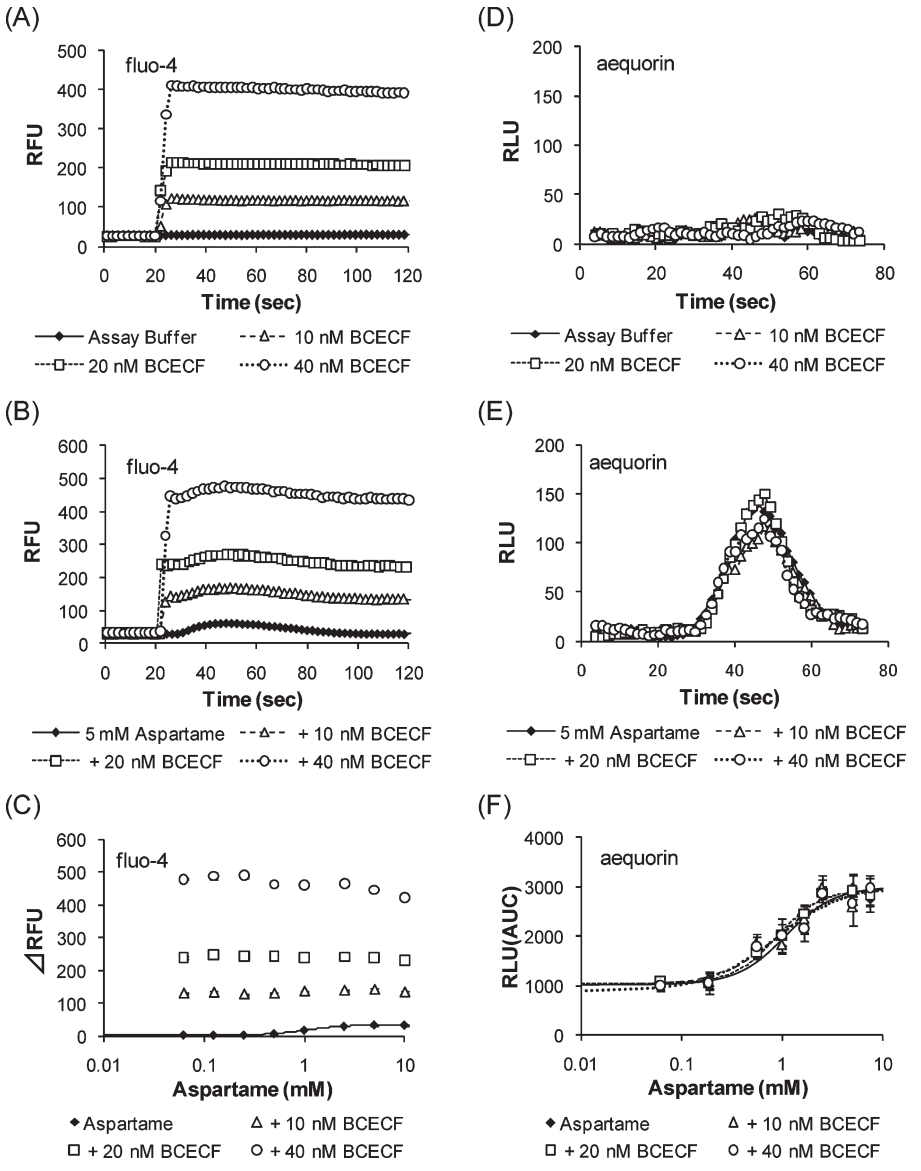

Figure 2. Comparison of the aequorin assay with the fluo-4 assay in the presence of BCECF. (A and B) Fluorescence intensities obtained using the fluo-4 assay when hT1R2/hT1R3-expressing cells were stimulated with assay buffer (A) or 5 mM aspartame (B) in the presence of the indicated concentrations of BCECF ($n = 3$). (D and E) Luminescence intensities obtained using the aequorin assay when hT1R2/hT1R3-expressing cells were stimulated with assay buffer (D) or 5 mM aspartame (E) in the presence of the indicated concentrations of BCECF ($n = 3-6$). (C and F) Dose−response curve obtained using the fluo-4 assay (C) or the aequorin assay (F) when hT1R2/hT1R3-expressing cells were exposed to aspartame in the presence of the indicated concentrations of BCECF. Each point indicates the mean ± SEM ($n = 3-6$).

FlexStation 3 microplate reader for an additional 55 s. The changes in luminescence intensity were monitored every 1.5 s and smoothed using a five-point moving average. The response from each well was expressed as RLU (relative light units) and calculated using the area under the curve (AUC). The responses were averaged from at least three wells receiving the same stimulus. To calculate the EC_{50} values and the signal/background ratios, plots of the amplitudes versus concentrations were fitted to the Hill's equation.

RESULTS

Application of the Luminescence-Based Assay to the Detection of Responses Mediated by the Human Sweet Taste Receptor. The luminescence-based assay was performed using a representative photoprotein, aequorin, to detect the taste receptor response. The results were compared with the fluorescence-based taste evaluation system using a conventional fluorescent intracellular Ca^{2+} indicator, fluo-4, to evaluate whether the luminescence-based assay was applicable as a sweet taste receptor assay.

The signals following sweet taste receptor activation in response to application of the artificial sweetener aspartame were measured using both assay systems. As a result, we succeeded in detecting sweetener-induced signaling using the luminescence-based assay; when the receptor was activated by aspartame, the luminescent intensity increased gradually and then declined over time in the same way as the fluorescent intensity monitored using the fluo-4 assay (Figure 1A,B). Moreover, the dose dependency of the response to aspartame was clearly observed using the

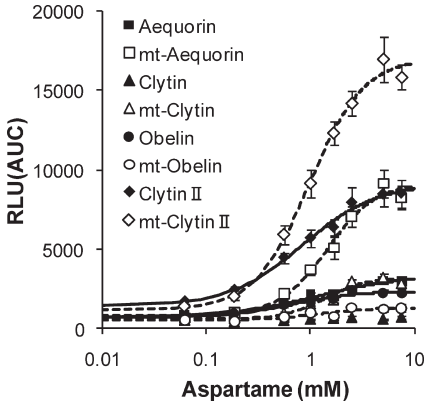

Figure 3. Comparison of the signals obtained from cytoplasmic and mitochondrial photoproteins. hT1R2/hT1R3-expressing cells were transiently transfected with each apophotoprotein expression plasmid and loaded with 10 μM coelenterazine. Each point indicates the mean ± SEM (n = 6). mt-, mitochondrial.

luminescence-based assay with aequorin (Figure 1D) and the fluo-4 assay (Figure 1C). The EC_{50} values measured in both assays were almost equal, that is, in the aequorin assay, EC_{50} was measured at 0.9 mM; in the fluo-4 assay, EC_{50} was measured at 1.7 mM, although the signal/background ratio obtained from the luminescence-based assay was lower than that in the fluorescence-based assay; that is, in the aequorin assay, signal/background = 2.8, and in the fluo-4 assay, signal/background = 12.0. This result indicates that the luminescence-based assay using a photoprotein is equally suitable to the conventional fluorescence-based taste evaluation system for detecting the response of the sweet taste receptor-expressing cells.

Evaluation of the Luminescence-Based Assay System in the Presence of an Artificial Fluorescent Substance. To investigate whether the luminescence-based assay could be applied to fluorescent samples, the luminescent signals from the photoprotein-transfected cells were measured in response to aspartame in the presence of a fluorescent substance, which was excited and detected at the wavelengths used in the fluo-4 measurements (Ex. 485 nm/Em. 525 nm).

We first selected BCECF, which is widely used as a fluorescent pH indicator, as a model fluorescent substance.[27] When the fluo-4-loaded cells were treated with 10−40 nM BCECF alone and the fluorescent intensity was monitored, a robust and dose-dependent rectangular fluorescence response was detected immediately after the BCECF solution was added (Figure 2A). Because the BCECF-derived fluorescence intensity remained approximately constant, it could be clearly distinguished from the response mediated by the sweet taste receptor, whose signal gradually increased after the addition of sweetener and declined with time (Figures 1A and 2B, 5 mM aspartame alone). In the fluorescence-based assay, the strong BCECF-derived fluorescence intensity interfered with detection of the cellular response to aspartame, and we failed to detect a dose−response relation in aspartame when more than 10 nM BCECF was added (Figure 2B,C).

In contrast, when the aequorin-expressing cells were treated with 10−40 nM BCECF and the luminescence intensity was monitored, the addition of BCECF did not result in nonspecific or dose-dependent luminescent signals. In addition, the time−response curves were nearly the same as the curve obtained after assay buffer application alone (Figure 2D), although BCECF was added at a high enough concentration to produce robust signals in the fluo-4 assay. Moreover, the cellular response to aspartame was precisely detected in the presence of BCECF using the aequorin assay, even when 40 nM BCECF was added to 5 mM aspartame (Figure 2E). The luminescence intensity of the aspartame-treated cells exhibited a dose dependence, and the EC_{50} values were approximately equal to each other, regardless of the presence of any of the BCECF concentrations tested (Figure 2F). Consequently, we confirmed that the luminescence-based assay using aequorin could be used as an appropriate evaluation system for the human sweet taste receptor in the presence of an artificial fluorescent substance.

Table 1. Comparison of Measured EC_{50} Values and Signal/Background Ratios from Cytoplasmic and Mitochondrial Photoproteins Using hT1R2/hT1R3-Expressing Cells[a]

photoprotein	EC_{50} (mM)	signal/background
aequorin	1.2	4.9
mt-aequorin	1.5	11.6
clytin	—[b]	—
mt-clytin	1.4	5.1
obelin	0.7	3.3
mt-obelin	1.2	2.6
clytin-II	0.8	6.6
mt-clytin-II	1.0	14.4

[a] The measured EC_{50} values and signal/background ratios were calculated by fitting the data to the Hill's equation. Signal/background = maximum signal/minimum signal. [b] —, value not determined.

Selection of Photoproteins for Use in the Luminescence-Based Assay of Sweet Taste Receptor-Expressing Cells. It is well-known that the luminescence intensity varies according to photoprotein type under the same experimental conditions. To optimize our luminescence-based assay using Ca^{2+}-sensitive photoproteins, we evaluated the luminescent properties of four photoproteins: aequorin, obelin, clytin, and clytin-II (Figure 3).

When human sweet taste receptor-expressing cells were transfected with expression plasmids encoding each apophotoprotein, that is, apoaequorin, apoobelin, apoclytin, and apoclytin-II, and then loaded with coelenterazine to form the active photoproteins, the luminescence intensities of the four photoproteins in response to aspartame could be detected in all cases except when clytin was used (Figure 3, closed symbols). The dose-dependent response to aspartame was detected using each indicator, and their EC_{50} values were all approximately 1 mM (Table 1). Among these photoproteins, only clytin-II produced a stronger luminescent signal intensity than aequorin (approximately three times higher).

We also evaluated the cellular response to aspartame using mitochondrial photoproteins because localization of photoproteins to the mitochondria has been reported to enhance luminescence in many GPCR assays.[20,23,28] The luminescence intensities produced by aequorin, clytin, and clytin-II were increased upon mitochondrial targeting without changes being observed in the EC_{50} values (Figure 3, open symbols; Table 1). Among the photoproteins, mitochondrial clytin-II yielded the highest signals, approximately five times higher than those produced by nonmitochondrially targeted aequorin, which was expressed in the cytoplasm (Figure 3). Mitochondrial clytin-II and mitochondrial aequorin generated the highest signal/background ratios, which were nearly equivalent to the ratio obtained using the

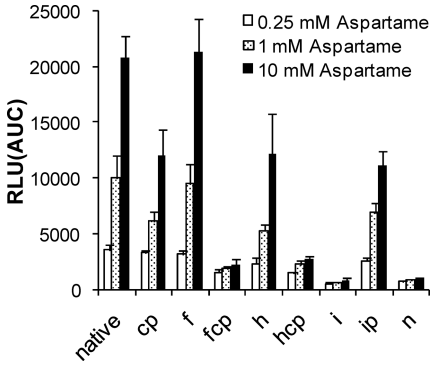

Figure 4. Comparison of coelenterazine and its derivatives in the mitochondrial clytin-II assay using hT1R2/hT1R3-expressing cells. The cells were loaded with native coelenterazine or each of eight analogues and stimulated with 0.25, 1, and 10 mM aspartame. Each bar indicates the mean ± SEM (n = 5−6).

fluo-4 assay (Figure 1C and Table 1). In this study, we adopted mitochondrial clytin-II for further validation.

Comparison between Coelenterazine and Its Derivatives as Luminescent Cofactors. Because of the differences in both Ca^{2+} sensitivities and luminescence properties, several types of coelenterazine derivatives have been synthesized[29,30] and used in photoprotein luminescence-based GPCR assays.[20,23,31] To select a suitable cofactor for our functional taste receptor assay, the luminescence intensities obtained from native coelenterazine and eight analogues were examined using mitochondrial clytin-II as the photoprotein.

The luminescence intensities in response to the application of aspartame varied among the coelenterazine derivatives used (Figure 4). The strongest signals were observed using native or coelenterazine f, and the signals obtained exhibited a dose-dependent response to aspartame. The cp, h, and ip derivatives also manifested dose-dependent responses to aspartame but yielded signals that were about half as intense as those from native and coelenterazine f. For this reason, native coelenterazine was selected for further experimentation.

Application of the Luminescence Assay in the Presence of an Autofluorescent Food-Derived Substance. The response of human sweet taste receptor-expressing cells was also measured by the mitochondrial clytin-II luminescence-based assay in the presence of fluorescent substances. First, we confirmed that this luminescence-based assay functioned as effectively as the aequorin assay in the presence of the model fluorescent substance BCECF (Figure 5A). Next, the cellular response was measured in the presence of a food-derived fluorescent substance. For this, we selected riboflavin (vitamin B_2) as a food-derived fluorescent substance, which is excited and detected at the wavelength used for fluo-4 measurements (Ex. 485 nm/Em. 525 nm).

When fluo-4-loaded cells were treated with 0.25−1.0 μg/mL of riboflavin and the fluorescence intensity was monitored, robust and dose-dependent rectangular fluorescence was detected immediately after the addition of the riboflavin solution (Figure 5B), similar to when BCECF was added (Figure 2A). The fluorescence derived from riboflavin was sufficiently strong to prevent detection of the sweet taste receptor response to aspartame (Figure 5C,D).

In contrast, when the mitochondrial clytin-II luminescence-based assay was used, the addition of riboflavin did not produce any nonspecific luminescence signals, and the time−response curves were nearly the same as the curves obtained using the assay buffer alone (Figure 5E). Furthermore, the luminescence intensities in response to aspartame did not change in the presence of any of the riboflavin concentrations added (Figure 5F,G).

DISCUSSION

In this study, we have established a luminescence-based assay system applicable to human sweet taste receptor-expressing cells. This assay system can be used to measure the sweet taste intensities of not only aspartame but also natural sweeteners, for example, sucrose (Figure S1 in the Supporting Information), which is about 100−200 times less sweet than aspartame.[32] Moreover, our results strongly indicate that photoprotein luminescence-based assays are suitable for the taste evaluation systems because these assays can overcome the limitation of ligands that contain fluorescent compounds (Figures 2 and 5).

Many researchers have developed cell-based assays for taste receptors that use fluorescent dyes as intracellular Ca^{2+} indicators.[26,33,34] However, these assays are limited because the cellular responses remain subject to interference from the surroundings (e.g., temperature, pH, and osmotic pressure). Moreover, detection of the fluorescent signals was often disturbed by the ligand because of the unsuitable characteristics of ligand (e.g., cytotoxicity, absorbance, and autofluorescence). Autofluorescence has been a serious limitation in previous assay systems, especially when samples derived from food materials were examined, because various fluorescent substances, for example, chlorophyll, vitamins, and Maillard reaction products, are contained in foods that can disturb the detection of the fluorescent signals produced by the fluorescent dyes. To address ligand autofluorescence, an electrophysiological assay, such as patch-clamp recording or the two-electrode voltage-clamp method using *Xenopus* oocytes, could be used. However, taste receptor-mediated responses are not necessarily converted to changes in membrane current. Therefore, the development of a high-throughput assay for taste receptors that is not affected by ligand autofluorescence is required. In this study, we have succeeded in establishing a sweet taste evaluation system for fluorescent ligands that uses Ca^{2+}-sensitive photoproteins as intracellular Ca^{2+} indicators.

Ca^{2+}-sensitive photoproteins, such as aequorin, obelin, and clytin, are derived from jellyfish and have been used as intracellular Ca^{2+} indicators in GPCR[19−22] and ion channel[23] assays. Because it is well-known that luminescent properties vary under the same experimental conditions depending on the type of photoprotein used, it is important to select the best agent for each assay system. Upon comparing the luminescence intensities of four types of photoprotein, the most robust signal was generated by clytin-II (Figure 3), which was isolated from the jellyfish *Clytia gregarium* as an isotype of clytin.[35] It was reported that although the luminescence capacity of clytin-II was almost the same as those of aequorin and clytin, the initial luminescence intensity of clytin-II triggered by Ca^{2+} was about five times higher, and thus, the signal-to-noise ratio of clytin-II was higher than those of aequorin and clytin.[35] Furthermore, localizing clytin-II and aequorin to the mitochondria resulted in improved signal/background ratios, which were nearly equivalent to the ratio obtained using the fluo-4 fluorescence-based assay (Figure 1C and Table 1). The location of the photoproteins did not result in changes in the EC_{50} values (Table 1); the mitochondrial photoproteins

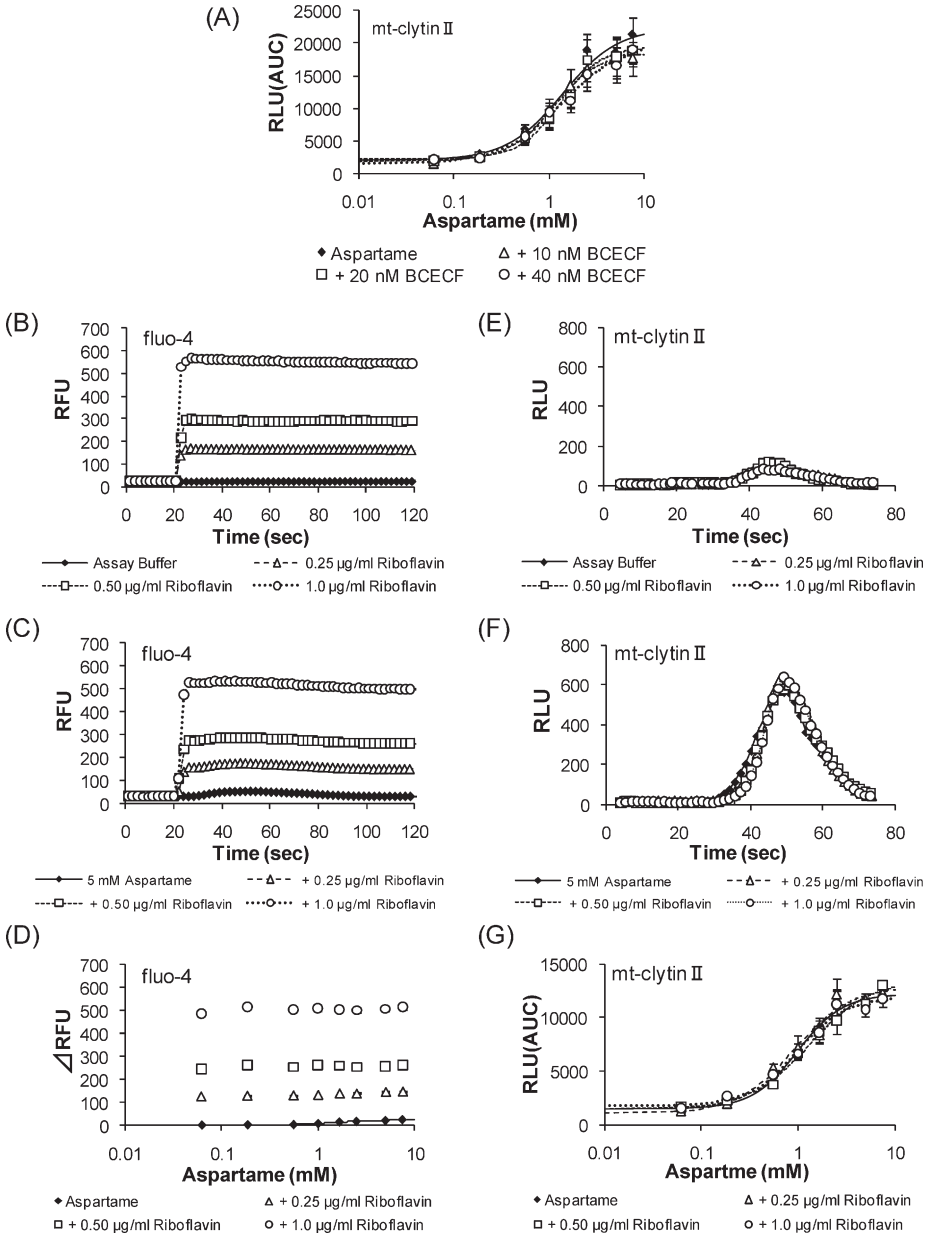

Figure 5. Comparison of the mitochondrial clytin-II assay with the fluo-4 assay in the presence of riboflavin, a fluorescent food substance. (A) Dose−response curve obtained using the mitochondrial clytin-II assay when hT1R2/hT1R3-expressing cells were exposed to aspartame in the presence of the indicated concentrations of BCECF. Each point indicates the mean ± SEM ($n = 6$). (B and C) Fluorescence intensities obtained using the fluo-4 assay when hT1R2/hT1R3-expressing cells were stimulated with assay buffer (B) or 5 mM aspartame (C) in the presence of the indicated concentrations of riboflavin ($n = 3$). (E and F) Luminescence intensities obtained using the mitochondrial clytin-II assay when hT1R2/hT1R3-expressing cells were stimulated with assay buffer (E) or 5 mM aspartame (F) in the presence of the indicated concentrations of riboflavin ($n = 3$). (D and G) Dose−response curve obtained using the fluo-4 assay (D) or the mitochondrial clytin-II assay (G) when hT1R2/hT1R3-expressing cells were exposed to aspartame in the presence of the indicated concentrations of riboflavin. Each point indicates the mean ± SEM ($n = 3$). The EC_{50} values were measured as 1.0 mM for aspartame, 0.9 mM for aspartame + 0.25 μg/mL riboflavin, 1.3 mM for aspartame + 0.50 μg/mL riboflavin, and 1.0 mM for aspartame + 1.0 μg/mL riboflavin using the mitochondrial clytin-II assay.

monitored the increases in intracellular Ca^{2+} concentration as accurately as their cytoplasmic counterparts. Both mitochondrial clytin-II- and mitochondrial aequorin-based assays should be suitable for use in sweet taste receptor evaluation systems.

We have demonstrated the advantage of the luminescence-based taste evaluation system by measuring the cellular responses to aspartame in the presence of the model fluorescent compounds BCECF and riboflavin. Because BCECF is widely used as an intracellular pH indicator and provides strong fluorescent signals at low concentration, we expected that BCECF would not introduce nonspecific cellular responses and would serve as a suitable model fluorescent substance. Actually, when we measured the BCECF signals using the fluo-4 assay, 10−40 nM BCECF produced approximately 4−14-fold higher signals than the maximum signals observed in response to aspartame (Figure 2A,B), and we failed to detect the actual sweet taste

signals to aspartame (Figure 2B,C). In contrast, BCECF did not produce nonspecific signals at the same concentrations in the aequorin assay (Figure 2D−F). This result indicates not only that the luminescence assay is useful for fluorescent ligands but also that BCECF is an ideal model fluorescent substance.

Riboflavin was selected as an example of a fluorescent substance that is commonly contained in food materials because it is known to possess fluorescent characteristics and is contained at high levels in various kinds of food, including milk, meat, fish, and leafy green vegetables.[36] When we measured the sweet taste signals developed in response to the addition of riboflavin at concentrations relevant to food-derived samples (0.25−1.0 μg/mL; e.g., leafy vegetables contain several μg/g edible portion), the response mediated by the sweet taste receptor could be detected using the mitochondrial clytin-II luminescence-based assay. We concluded that riboflavin did not modulate activation of the sweet taste receptor within the range tested (Figure 5E−G). However, we were unable to evaluate this phenomenon using the conventional fluorescence-based assay system (Figure 5B−D).

Recently, artificial chemical compounds that have sweet taste-enhancing effects have been identified using both cell-based assays and human taste tests.[37] This result suggests that cell-based assays could function together with the human sensory test as a taste evaluation system. In our data, the EC_{50} values of aspartame, saccharin, and sucrose were measured as 1.0, 0.3, and 104 mM, respectively (Figure 5G and Figure S1 in the Supporting Information). This result is well correlated with the data from human taste tests showing that aspartame and saccharin were about 100−200 and 200−400 times as sweet as sucrose, respectively.[32] Furthermore, cell-based assays are very useful not only for screening taste modulators but also for analyzing the mechanism of taste modulation.[38−41] In this study, we used the luminescence-based assay only for evaluating the sweet taste receptor, but the assay can also be applied to evaluate other taste GPCRs, such as the umami and bitter taste receptors, and ion channels, such as TRPV1, which senses hot taste.[23] Many classes of taste modulators are likely to be contained in food materials, and this novel taste evaluation system would be a powerful tool to elucidate these food-derived modulators even at an industrial level. Further investigation is in progress to characterize new taste modulators in foods.

■ ASSOCIATED CONTENT

Supporting Information. Figure of the dose−response curves to sweeteners using the luminescence assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AUC, area under the curve; BCECF, 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein; mt-, mitochondrial; ΔRFU, Δ relative fluorescence units; RLU, relative light units

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