Food Bioscience 50 (2022) 102186

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Red algae-derived isofloridoside activates the sweet taste receptor T1R2/T1R3

Miku Akishino [a], Yuko Aoki [b], Hajime Baba [b], Makio Asakawa [d,1], Yoichiro Hama [b,c,e], Susumu Mitsutake [a,c,e,*]

[a] *Graduate School of Advanced Health Sciences, Saga University, Saga, Japan*
[b] *Graduate School of Agriculture, Saga University, Saga, Japan*
[c] *Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, Saga, Japan*
[d] *Department of Food Science, Faculty of Education, Kumamoto University, Kumamoto, Japan*
[e] *The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan*

ARTICLE INFO

Keywords:
GPCR
Taste receptor
T1R2
T1R3
Floridoside
Pyropia yezoensis

ABSTRACT

Isofloridoside (IF) is a naturally occurring galactosylglycerol compound with a refreshing sweet taste. It is found in red algae, where it acts as a photosynthetic intermediate. However, the mechanism by which IF elicits sweetness has not been determined. In this study, we investigate the interaction between IF and the sweet taste receptor T1R2/T1R3. We show that IF causes an increase in intracellular Ca^{2+} concentration and an increase in Erk phosphorylation in T1R2/T1R3-expressing HEK293T cells, indicating that IF interacts with T1R2/T1R3. IF also activates endogenously expressed T1R2/T1R3 in mouse small intestinal endocrine L-cells, and promotes the secretion of the incretin glucagon-like peptide-1 (GLP-1). *In silico* docking simulations of IF and T1R2/T1R3 predict that IF forms hydrogen bonds with Tyr103 in the extracellular Venus flytrap domain of T1R2. This result is consistent with the binding mode of many other sweet-tasting molecules. We found that IF have the potential as a new alternative sweetener.

1. Introduction

We sense sweetness via a heterodimeric receptor complex composed of taste receptors type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3), which are present in the cells of our taste buds. T1R2 and T1R3 are class C G-protein coupled receptors (GPCRs), but are also classified as metabotropic glutamate receptors (mGluRs), $GABA_B$ receptors, and V2R pheromone receptors (Chandrashekar et al., 2006). The receptors are only responsive to sweet-tasting substances after forming the T1R2/T1R3 heterodimer (Nelson et al., 2001). When the heterodimer binds to sweet-tasting substances, it activates and transmits a sweet taste signal to the brain. Both T1R2 and T1R3 receptors are composed of a 7-transmembrane domain (TMD), a cysteine-rich domain (CRD), and an N-terminal domain (NTD), which contains a large extracellular domain. An extended pocket-shaped region on the NTD, called the Venus flytrap domain (VFD), plays an important role in the recognition and binding of a wide variety of sweet-tasting substances, including proteins, amino acids, small molecules, and various high-molecular weight compounds (DuBois, 2016; Nuemket et al., 2017).

GPCRs are associated with heterotrimeric G-proteins; upon receptor stimulation, the G-protein dissociates into its component subunits (α, β, and γ), which transmit downstream signals within the cell. Taste bud cells express both T1R2/T1R3 and the characteristic heterotrimeric G-protein gustducin (Stone et al., 2007). The α-subunit of gastducin ($G\alpha_{gust}$) is required for T1R2/T1R3 signal transduction. T1R2 and T1R3 are widely distributed in the digestive tract, pancreas, brain, and have many other roles besides taste recognition (Kohno, 2017); (Lee & Cohen, 2014) $G\alpha_{gust}$ is expressed in the stomach, duodenum, pancreatic ducts, intestinal and endocrine cells (Stone et al., 2007).

Low-calorie artificial sweeteners, such as acesulfame K (Ace K) and aspartame, are widely used in foods and in the management of type 2 diabetes mellitus (Li et al., 2011). However, there are concerns that artificial sweeteners may stimulate lipogenesis and cause addiction and carcinogenesis (Simon et al., 2013). Therefore, the development of new natural sweeteners with improved safety profiles is required. In this

* Corresponding author. Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, Honjo-machi 1, Saga, 840-8502, Japan.
E-mail address: susumumi@cc.saga-u.ac.jp (S. Mitsutake).
[1] Present: Specified Nonprofit Corporation of Nobel Application of Plant Resources, Kumamoto.

https://doi.org/10.1016/j.fbio.2022.102186
Received 1 September 2022; Received in revised form 17 October 2022; Accepted 5 November 2022
Available online 9 November 2022
2212-4292/© 2022 Elsevier Ltd. All rights reserved.

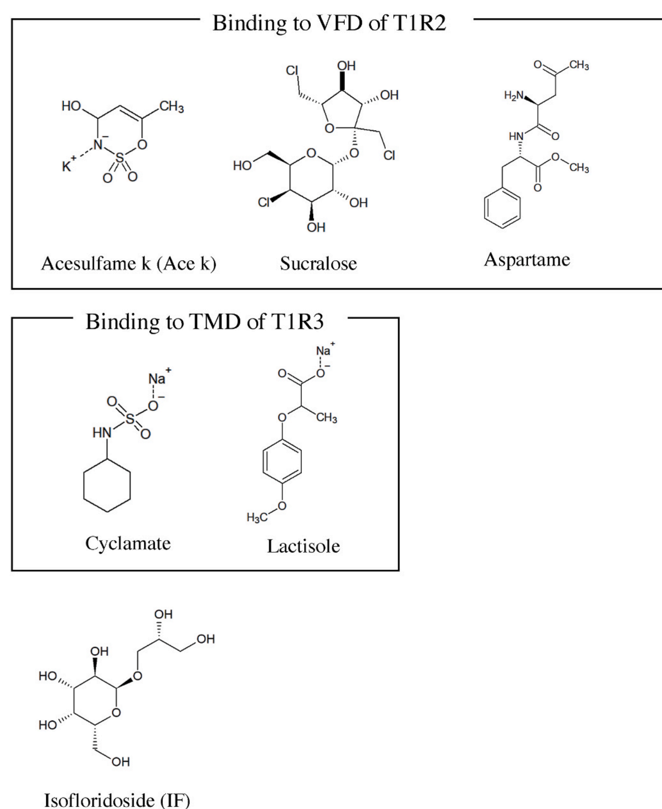


Fig. 1. Structures of IF, sweet-tasting substances, and sweetness inhibitor. Structures of sweeteners (Ace K, sucralose, aspartame, and cyclamate), sweetness inhibitor (Lactisole), and isofloridoside (IF). Ace K, sucralose, and aspartame bind to the large, extracellular VFD of T1R2 (Masuda et al., 2012), while cyclamate binds to the TMD of T1R3 (Winnig et al., 2007). Interestingly, lactisole also binds to the TMD of T1R3, but suppresses sweetness (Galindo-Cuspinera et al., 2006).

study, we focus on isofloridoside (IF), a low molecular weight carbohydrate from the red algae *Pyropia yezoensis* (Colin & Guéguen, 1930), (H. Noda, H, Abo, & Horiguchi, 1981) which is commonly used to produce nori. IF is a galactosylglycerol (a galactose molecule is connected to a glycerol backbone) (Putman & Hassid, 1954) and acts as a photosynthetic intermediate. (Kirst & Bisson, 1979); (Bean & Hassid, 1955). It has been reported that IF reaches the large intestine without being digested or absorbed. It is therefore a zero-calorie molecule that is not absorbed by the small intestine for use as energy. In addition, it acts as a prebiotic that improves the intestinal environment. (Muraoka et al., 2008); (Ishihara et al., 2010) In our experience, IF has a sweet taste with a refreshing quality. However, the mechanism by which IF elicits sweetness and its interaction with T1R2/T1R3 have not been investigated. Here, we elucidate the physiological action of IF as a sweetener.

2. Materials and Methods

2.1. Materials

Polyethylenimine (PEI) and lactisole were obtained from Polyscience Inc. (Warrington, PA, USA) and Cayman Chemical Company (Ann Arbor, MI, USA), respectively. Dried sheets of nori were a kind gift from Kumamoto Prefectural Federation of Fisheries Cooperative. Acesulfame K, HHBS, and other chemicals were purchased from FUJIFILM Wako Pure Chemical Corporation, (Osaka, Japan), unless specified otherwise. All reagents were of the highest purity commercially available.

2.2. Preparation of isofloridoside

Isofloridoside (IF) was prepared as previously reported (Simon-Colin et al., 2002). Briefly, dried sheets of nori were disrupted in a MeOH/$CHCl_3$/H_2O (12/5/3, v/v/v) mixture, and the supernatant was obtained by centrifugation for 5 min at 5000×*g*. The supernatant was evaporated, and the resulting residue was redissolved in distilled water and partitioned by passing through a column of cationic resin (AG®50-X8 hydrogen form, BioRad, Berkeley, CA, USA) and anionic resin (AG®1-X8 hydroxide form, BioRad) to remove ionic molecules. The passed fraction was dried and dissolved in distilled water. Ethanol was added to a final concentration of approximately 89%. The solution was left to sit at 4 °C for 2 weeks to induce the crystallization of IF. The isolated crystals were identified as IF by nuclear magnetic resonance (NMR) analysis, and their purity (>95%) was assessed by GC-MS, as described by U. Karsten et al. (Karsten & West, 2000)

2.3. Cell culture

The HEK293T cell line was obtained from Riken BRC, and cultured in Dulbecco's modified Eagle's medium (D-MEM, Sigma Aldrich Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum. The STC-1 (CRL-3254) cell line was obtained from the American Type Culture Collection (ATCC) and cultured in D-MEM (Sigma) supplemented with 5% fetal bovine serum and 5% horse serum (Rindi et al., 1990).

2.4. Plasmid design

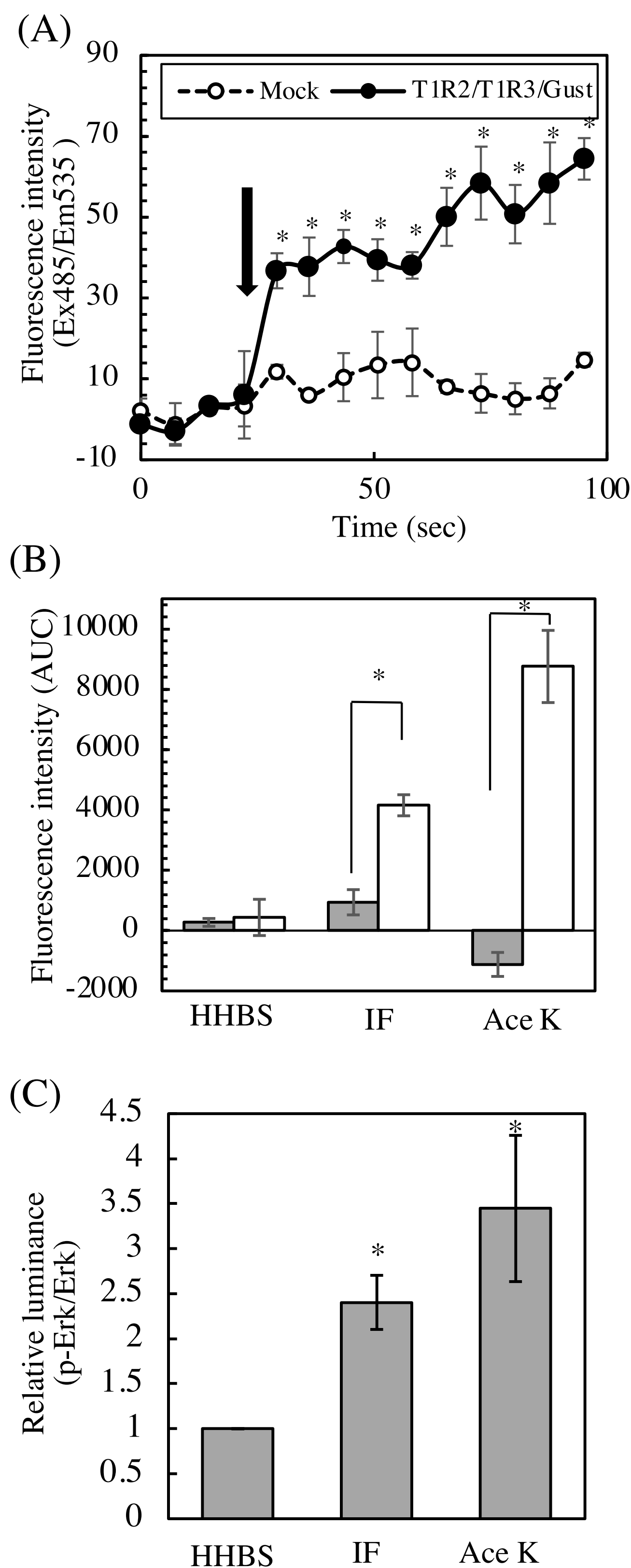
The signal sequence (1–57) of Human T1R2 (NM_152232) was removed and cloned into the p3XFLAG-CMV23 plasmid (Sigma) to construct a T1R2 expression vector with a preprotrypsine signal sequence and a FLAG epitope tag at its N-terminus. The signal sequence (1–90) of IL6 (NF_011640) was cloned into the pcDNA3.1 nV5 plasmid (Invitrogen), ahead of the sequence encoding the V5 epitope, to construct pcDNA3.1 IL6-nV5. The T1R3 sequence, excluding the signal sequence (1–60), was cloned into this vector to construct a T1R3 expression vector with the signal sequence of IL6 and a V5 epitope tag at its N-terminus. Human gustducin (NM_001102386) was also cloned into pcDNA3.1nV5 to construct an expression vector.

2.5. Western blotting

The T1R2, T1R3, and gustducin expression plasmids were transfected into HEK293T cells using polyethylenimine (PEI) as previously reported (Nagasawa et al., 2018). After 24 h, the medium was changed to serum-free DMEM. The cells were cultured for another 6 h and then treated with 10 mM IF, Ace K, or HHBS for 10 min. The cells were harvested and lysed with a buffer composed of 50 mM Tris-HCl buffer, pH 7.5, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.25% sodium deoxycholate, 1 mM EDTA, PhosSTOP™ (Roche Diagnostics, Basel, Switserland), and Complete™ EDTA-free (Roche Diagnostics). The proteins were separated by SDS-PAGE, and western blotting was performed as previously described (Nagasawa et al., 2020). Chemiluminescence was monitored using LuminoGraph I (ATTO Corp., Tokyo, Japan), and the signals were quantified by CS Analyzer (ATTO Corp.) in accordance with the manufacturer's instructions. The primary antibodies used were: phosphor-p44/42 MAP kinase antibody and p44/42 MAP kinase antibody (Cell Signaling Technology, Danvers, MA, USA). The secondary antibodies used were: anti-mouse F (ab')-HRP and anti-rabbit F (ab')-HRP (Medical & Biological Laboratories, Tokyo, Japan).

2.6. Measurement of intracellular Ca^{2+} concentration

The intracellular Ca^{2+} concentration was measured using the calcium indicator Calbryte 520 a.m. (AAT Bioquest, Sunnyvale, CA, USA), in accordance with the manufacturer's instructions (Nagasawa et al.,



(caption on next column)

Fig. 2. IF-induced intracellular Ca^{2+} elevation in a sweet taste receptor T1R2/T1R3-dependent manner.
(A) Changes in intracellular Ca^{2+} were measured using the Ca^{2+} probe Calbryte™ 520-AM. Expression plasmids for T1R2, T1R3, and gustducin, or the pCMV23 plasmid (mock), were introduced into HEK293T cells. On the following day, CalbryteTM 520-AM was introduced into the cell. While measuring the fluorescence, IF was added to a final concentration of 10 mM using an injector (arrow). Full details are described in the Experimental section. Error bars represent SD from 4 independent experiments; *$p < 0.05$ versus *Mock*. (B) Area under the curve (AUC) values were calculated from experiment A. The white bars indicate HEK293T cells expressing T1R2, T1R3, and gustducin; The grey bars indicate HEK293T cells transfected with mock plasmid. Error bars represent SD; *$p < 0.05$ versus *Mock*. (C) Detection of Erk phosphorylation by western blotting, a downstream event of T1R2/T1R3 activation. The expression plasmids for T1R2, T1R3, and gustducin were transfected into HEK293T cells. After addition of IF, Ace K, or HHBS, phosphorylation of Erk was examined by western blotting. The intensity of the signals were quantified and expressed as the ratio of phosphorylated Erk/Erk. Error bars represent SD from 4 independent experiments; *$p < 0.05$ versus HHBS treatment. All statistical analysis was done using Student's *t*-test.

2020). The fluorescence was measured using an Infinite F200 PRO microplate reader (TECAN Group Ltd., Switzerland) with an excitation wavelength of 488 nm and an emission wavelength of 510 nm. Ace K (10 mM), IF (10 mM),HHBS, or lactisole (4 mM) were added using an injector attached to the device at indicated times.

2.7. qPCR experiment

The total RNA of STC-1 cells was extracted using TRIzol™ reagent (Themo Fisher Scientific Inc., Waltham, MA, USA) and the cDNA was prepared using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan) in accordance with the manufacturer's instructions. qPCR analysis was performed using Thunderbird SYBR qPCR mix (Toyobo Co., Ltd.) on a Light Cycler 96 System (Roche Diagnostics). The following primers were used: T1R2, forward 5′-GTCCGCTGCACCAAGCA-3′ and reverse 5′-GTTCGTCGAAGAAGAGCTGGTT-3'; T1R3, forward 5′-TCAGAGCTTGCCCGCATTACAG-3′ and reverse 5′-TGTGCGAAGAAGGATGGA-3'; GAPDH, forward 5′-CATCACTGCCACCCAGAAGACT-3′ and reverse 5′-ATGCCAGTGAGCTTCCCGTTCA-3'.

2.8. Enzyme-linked immunosorbent assay of GLP-1

STC-1 cells were cultured in 24-well plates. The medium was changed to DMEM, without glucose (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) and supplemented with 1 mM sodium pyruvate and 4 mM L-glutamine for 6 h. The culture medium was collected 60 min after the addition of Ace K (10 mM), IF (10 mM), α−linoleic acid (ALA, 25 μM) or HHBS. The amount of GLP-1 in the medium was determined using a GLP-1 ELISA kit (FUJIFILM Wako Pure Chemical Corporation), in accordance with the manufacturer's instructions (Nagasawa et al., 2020).

2.9. In silico simulation

We constructed 3D-structure models of T1R2 (NP_689418.2), T1R3 (NP_689414.2), and the T1R2/T1R3 heterodimer using AlphaFold 2.0 (Jumper et al., 2021). The models were visualized using PyMol Graphics System, version 2.5 (Schrodinger, Inc.) (https://pymol.org/2/). AutoDock 4.2 (Forli et al., 2016) (https://autodock.scripps.edu/download-autodock4/) was used to build the docking models for IF and T1R2/T1R3. Molegro Molecular Viewer 2.5 (https://molegro-molecular-viewer.software.informer.com/2.5/) was used to analyze the binding site and hydroxyl bond length of the obtained docking model (Thomsen & Ghristensen, 2006).

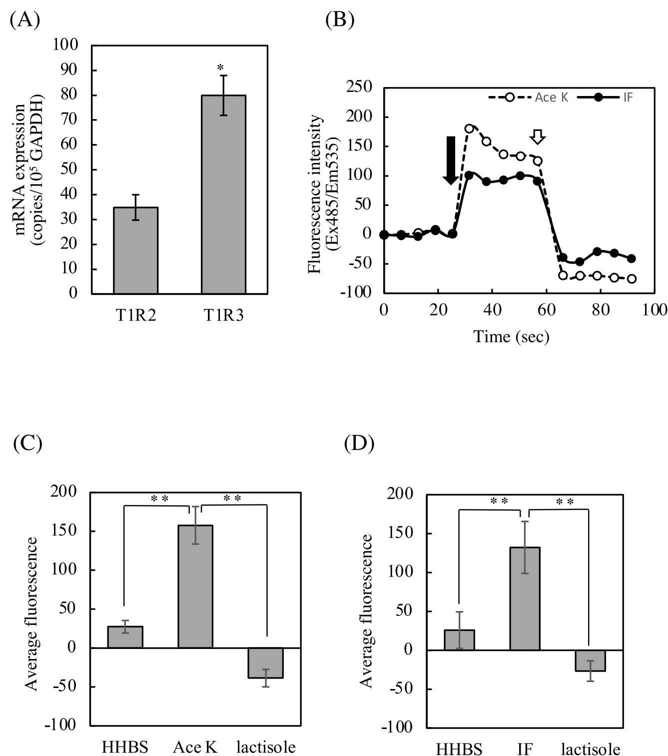


Fig. 3. IF-induced intracellular Ca^{2+} elevation via the activation of endogenous T1R2/T1R3 in STC-1 cells.
(A) Expression levels of T1R2 and T1R3 in STC-1 cells were examined by qPCR as described in the Experimental section. $*p < 0.05$ (B) Changes in intracellular Ca^{2+} were measured using the Ca^{2+} probe Calbryte™ 520-AM in STC-1 cells, as described in the Experimental section. The black arrow represents the time point of IF and Ace K addition and the white arrow represents the time point of lactisole addition. In experiment B, the average fluorescence intensity during 20 s after from the addition of Ace K, IF, or lactisole, was calculated and plotted (C and D). Error bars represent SD from 4 independent experiments; $**p < 0.01$. All statistical analysis was done using Student's *t*-test.

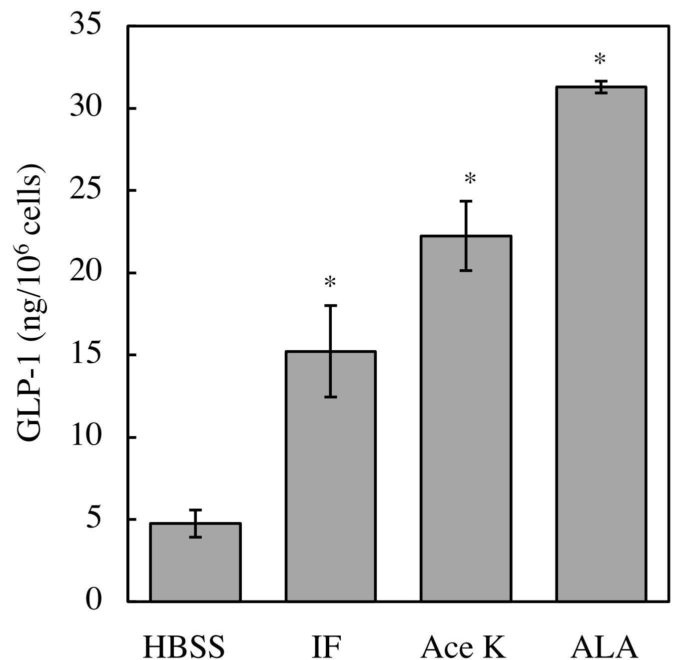


Fig. 4. IF promotes the secretion of GLP-1 from STC-1 cells
One hour after adding IF, Ace K, or ALA to STC-1 cells, the medium was collected and the amount of GLP-1 was quantified using ELISA, as described in the Experimental section. Error bars represent SD from 3 independent experiments; $*p < 0.05$. Statistical analysis was done using Student's *t*-test.

3. Results

3.1. IF-induced intracellular Ca^{2+} elevation in a sweet taste receptor T1R2/T1R3-dependent manner

The T1R2/T1R3 sweet taste receptor interacts with various molecules, which bind to different regions of the receptor (Fig. 1). The binding of molecules with various structures to different regions of the receptor makes it challenging to find common structural motifs. IF is a natural product that has a refreshing sweet taste. However, the mechanism by which IF transmits sweetness has not been clarified. In this study, we examine the physiological action of IF.

To examine whether IF acts on T1R2/T1R3, we measured the change in intracellular Ca^{2+} in cultured cells as described in Materials and Methods. A rapid increase in intracellular Ca^{2+} was observed after addition of IF (Fig. 2A, arrow). However, almost no increase in Ca^{2+} concentration was observed in the cells that were transfected with a control plasmid. IF therefore increased the intracellular Ca^{2+} in a receptor T1R2/T1R3-dependent manner (Fig. 2A and B). Ace K, a well-known sweetener, also increased the intracellular Ca^{2+} concentration in a T1R2/T1R3-dependent manner (Fig. 2B) and to a higher extent than IF. Ace K is an artificial sweetener that is 200 times sweeter than sucrose and known to be a potent T1R2/T1R3 activator. In addition, IF increased the phosphorylation of Erk, which is a downstream component of the T1R2/T1R3 signaling pathway that is phosphorylated upon receptor activation (Fig. 2C). In summary, IF increased the levels of intracellular Ca^{2+} and Erk phosphorylation, which suggests that IF interacts with T1R2/T1R3 to transmit sweet taste.

3.2. IF-induced intracellular Ca^{2+} elevation via the activation of endogenous T1R2/T1R3, and promotes the secretion of GLP-1 in STC-1 cells

In recent years, it has become clear that sweet taste receptors are expressed in various organs of the body and play important roles (Kohno, 2017); (Lee & Cohen, 2014). In the gastrointestinal tract, they work as nutrition sensors and are critical to the secretion of incretins, a group of metabolic hormones (Jang et al., 2007). Incretins have various functions, including the suppression of appetite and the stimulation of insulin secretion, many of which have anti-obesity and anti-diabetic effects. For instance, the incretin GLP-1 has potent anti-diabetic activity. Inhibitors of the GLP-1 degrading enzyme DPP4 are marketed as treatments for type 2 diabetes mellitus (Cahn et al., 2016).

To explore the physiological function of IF in the gastrointestinal tract, we used mouse small intestinal endocrine L-cells (STC-1 cells), which are capable of secreting GLP-1. Although a significant difference in expression level was observed between T1R2 and T1R3, we confirmed that the STC-1 cells expressed both T1R2 and T1R3 at sufficient levels (Fig. 3A). The addition of both IF and Ace K caused a rapid increase in intracellular Ca^{2+} levels (Fig. 3B, black arrow). Lactisole, a naturally occurring carboxylic acid possessing a hydrophobic phenoxyl group, is known to inhibit sweetness and the umami taste by binding to the TMD of T1R3 (Galindo-Cuspinera et al., 2006) (Fig. 1). The addition of lactisole to the same cells caused a rapid decrease in intracellular Ca^{2+} levels (Fig. 3B, white arrow; Fig. 3C and D). These results clearly demonstrate that IF and Ace K increase intracellular Ca^{2+} levels by interacting with T1R2/T1R3, which is endogenously expressed in STC-1 cells.

One hour after the addition of IF, we found that the level of GLP-1 in the medium had significantly increased (Fig. 4). Free fatty acid receptor 4 (FFAR4) is known as a major receptor involved in GLP-1 secretion in the gastrointestinal tract (Hirasawa et al., 2005). IF acted on T1R2/T1R3 to induced GLP-1 secretion with a comparable intensity to ALA induced

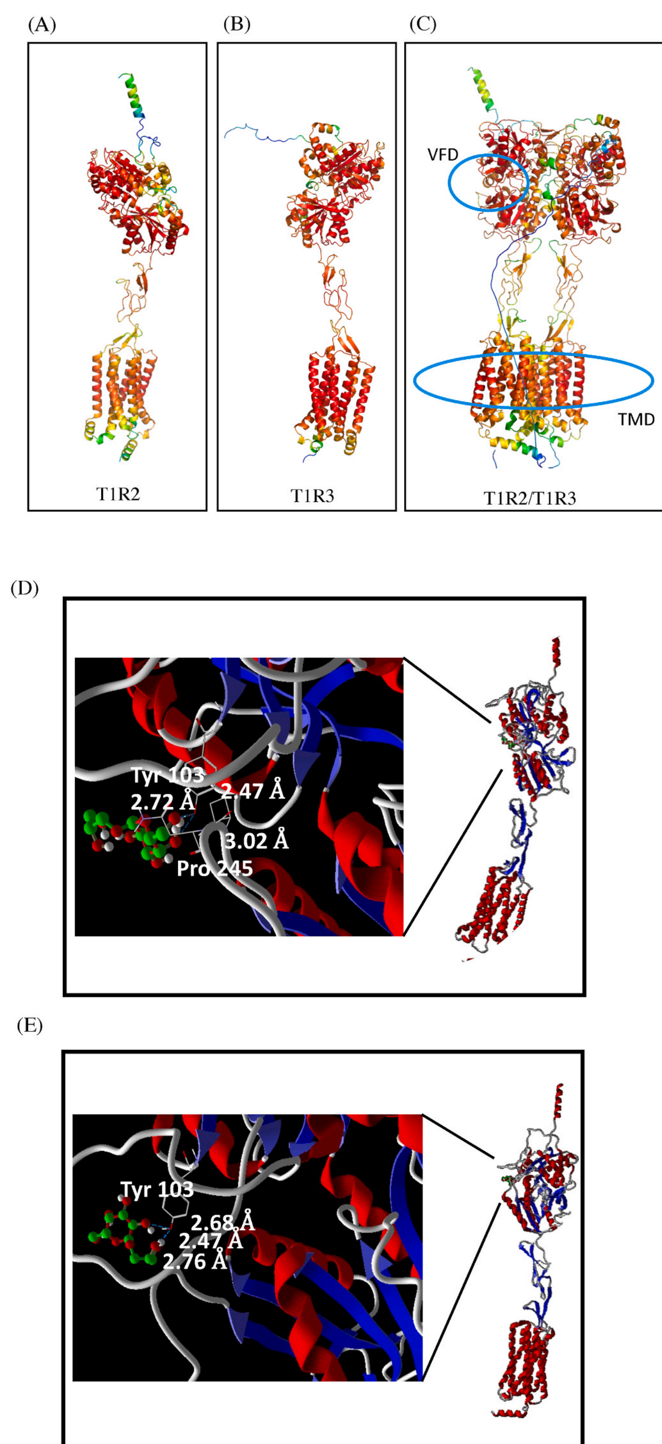


Fig. 5. Predicted 3D structure and docking simulation of T1R2/T1R3 and IF The 3D structures of T1R2 (A), T1R3 (B), and the T1R2/T1R3 complex (C) were predicted with AlphaFold 2.0 and visualized with PyMOL. These models are colored according to the pLDDT score: high pLDDT scores are shown in red. The docking models of IF with each 3D receptor model were prepared by AutoDock, and the details of the interactions were analyzed with Molegro Molecular Viewer. We obtained two docking models (D and E). IF forms two hydrogen bonds with Tyr103 (2.72 Å and 2.47 Å) in model D, and one hydrogen bond with Pro245 (3.02 Å), and three hydrogen bonds with Tyr103 (2.68 Å, 2.47 Å, and 2.76 Å) in model E. Full details of the *in silico* simulations are described in the Experimental section. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

FFAR4 activation (Fig. 4).

3.3. A docking simulation of T1R2/T1R3 and IF showed that IF forms hydrogen bonds with Tyr103 and Pro245 of the VFD of T1R2

Finally, we performed *in silico* docking simulations to determine how IF interacts with T1R2/T1R3. We used AlphaFold 2.0 to predict the structure of T1R2, T1R3, and the T1R2/T1R3 complex (Fig. 5A, B, C). The pLDDT values, which indicate the reliability of the 3D model, were 85 for T1R2, 86 for T1R3, and 79 for the T1R2/T1R3 complex model. Regions with high pLDDT values, which correspond to high reliability, are shown in red in the figure. We were able to construct a highly reliable model in the region where sweet-tasting compounds are known to bind (VFD of T1R2 and TMD of T1R3) (Fig. 5C). A docking model of IF and 3D models of the receptor were prepared by AutoDock, and the details of the interactions were analyzed by Molegro Molecular Viewer. Multiple binding modes were predicted by the docking simulation, all of which involve IF binding to the extracellular domain (VFD) of T1R2. For example, IF forms two hydrogen bonds with Tyr103 and one hydrogen bond with Pro245 in the binding mode shown in Fig. 5D, and three hydrogen bonds with Tyr103 in the binding mode shown in Fig. 5E. Many of the predicted hydrogen bonds are 3 Å or less in length, which is consistent with experimental observations of hydrogen bond length. Comparing the two models, the model in Fig. 5E forms a stronger hydrogen bond, which can be consider stable. Many other sweet-tasting molecules are also predicted to bind to the VFD of T1R2. Ace K is expected to form hydrogen bonds with Asp307, Asp142, Asp278, Ser165, Tyr103, and Pro277; sucralose with Glu302, Asp142, Tyr103, Asp278, Asp307, and Pro277; and aspartame with Glu302, Ser144, Asp142 Tyr103, and Asp278 (Masuda et al., 2012). Tyr103 plays an important role in all of these interactions. It is therefore encouraging that Tyr103 is involved in the IF binding modes predicted by the docking simulation.

4. Discussion

In this study, we determined that IF, found in *Pyropia yezpensis,* interacts with the sweet taste receptor T1R2/T1R3. This elucidates, for the first time, the physiological mechanism by which IF acts as a sweetener. It has been reported that IF is not digested or absorbed; it mostly reaches the large intestine, where it acts as a prebiotic, improving the intestinal environment. These suggest that we cannot use IF as nutrition and convert it into energy. Although it remains to be studied in detail, IF has a potential as a zero-calorie natural sweetener. Additionally, we found that IF promotes GLP-1 secretion via T1R2/T1R3 activation. Because the majority of IF reaches the small intestine in its original form, it is expected to interact with T1R2/T1R3 in the small intestinal endocrine L-cells, thereby promoting the secretion of GLP-1. The induction of GLP-1 secretion with IF would be beneficial in treating obesity and type 2 diabetes mellitus. Previously, we reported that phytosphingosine, found in yeast cell membranes, and teadenol A, found in microbially fermented tea, activate FFAR4 to induce GLP-1 secretion (Nagasawa et al., 2018, 2020). Both of these compounds are zero-calorie molecules. There may be many more molecules that activate incretin signaling and maintain systemic homeostasis. These molecules may be part of our diet without our being aware of their beneficial properties.

We performed docking simulations of IF and T1R2/T1R3, and showed that IF forms hydrogen bonds with Tyr103 and Pro245 of the VFD of T1R2. As detailed above, other sweeteners are also predicted to form hydrogen bonds with Tyr103. Tyr103 may therefore be an important residue in the receptor interaction with sweet-tasting substances. The remaining amino acids in the binding interaction are dependent on the ligand. These differences may contribute to the subtle differences in sweetness among substances. For instance, Ace K has a strong sweetness, while IF has a low sweetness with a refreshing quality.

In summary, we determined that IF interacts with T1R2/T1R3 to elicit a sweet taste and to induce GLP-1 secretion in the small intestine.

These results indicate that IF holds promise as an alternative sweetener for patients with diabetes and obesity.

Author contributions

M. Akishino, **Y. Aoki**, **H. Baba**: Investigation, Writing – Original Draft; **M. Asakawa**: Resources, Methodology; **Y. Hama**; Resources, Supervision; **S. Mitsutake**: Conceptualization, Writing – Review & Editing, Supervision, Project administration. All authors approved the final version of the manuscript to be published.

Ethics statements

Our research did not include any human subjects and animal experiments.

Declaration of competing interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Reaserch (C) 20K05927 from JSPS KAKENHI, Japan. We also thank Analytical Research Center for Experimental Sciences, Saga University for *in silico* analysis using AlphaFold 2. We thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Abbreviations

IF	isofloridoside
Ace K	acesulfame K
HHBS	Hanks' buffer with 20 mM HEPES
VFD	Venus flytrap domain
TMD	transmembrane domain
ALA	α-linolenic acid
GLP-1	glucagon-like peptide-1
CRD	cysteine-rich domain
NTD	N-terminal domain
GPCR	G-protein coupled receptor

References

Bean, R. C., & Hassid, W. Z. (1955). Assimilation of $C^{14}O_2$ BY a photosynthesizing red alga, iridophycus flaccidum. *Journal of Biological Chemistry, 212*(1), 411–425.

Cahn, A., Cernea, S., & Raz, I. (2016). An update on DPP-4 inhibitors in the management of type 2 diabetes. *Expert Opinion on Emerging Drugs, 21*(4), 409–419.

Chandrashekar, J., Hoon, M. A., Ryba, N. J., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature, 444*(7117), 288–294.

Colin, H., & Guéguen, E. (1930). La constitution du principe sucre de Rhodymenia palmata. *Comptes Rendus de Academie Sciences, 191*, 163–164.

DuBois, G. E. (2016). Molecular mechanism of sweetness sensation. *Physiology & Behavior, 164*(Pt B), 453–463.

Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols, 11*(5), 905–919.

Galindo-Cuspinera, V., Winnig, M., Bufe, B., Meyerhof, W., & Breslin, P. A. S. (2006). A TAS1R receptor-based explanation of sweet 'water-taste. *Nature, 441*(7091), 354–357.

Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., Sugimoto, Y., Miyazaki, S., & Tsujimoto, G. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nature Medicine, 11*(1), 90–94.

Ishihara, K., Oyamada, C., Sato, Y., Suzuki, T., Kaneniwa, M., Kunitake, H., & Muraoka, T. (2010). Prebiotic effect of glycerol galactoside isolated from color-faded nori in rats. *Fisheries Science, 76*(6), 1015–1021.

Jang, H.-J., Kokrashvili, Z., Theodorakis Michael, J., Carlson Olga, D., Kim, B.-J., Zhou, J., Kim Hyeon, H., Xu, X., Chan Sic, L., Juhaszova, M., et al. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proceedings of the National Academy of Sciences, 104*(38), 15069–15074.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature, 596*(7873), 583–589.

Karsten, U., & West, J. A. (2000). Living in the intertidal zone - seasonal effects on heterosides and sun-screen compounds in the red alga Bangia atropurpurea (Bangiales). *Journal of Experimental Marine Biology and Ecology, 254*(2), 221–234.

Kirst, G. O., & Bisson, M. A. (1979). Regulation of turgor pressure in marine algae: Ions and low-molecular-weight organic compounds. *Functional Plant Biology, 6*(4), 539–556.

Kohno, D. (2017). Sweet taste receptor in the hypothalamus: A potential new player in glucose sensing in the hypothalamus. *The Journal of Physiological Sciences, 67*(4), 459–465.

Lee, R. J., & Cohen, N. A. (2014). Bitter and sweet taste receptors in the respiratory epithelium in health and disease. *Journal of Molecular Medicine, 92*(12), 1235–1244.

Li, X., Servant, G., & Tachdjian, C. (2011). The discovery and mechanism of sweet taste enhancers. *Biomolecular Concepts, 2*(4), 327–332.

Masuda, K., Koizumi, A., Nakajima, K., Tanaka, T., Abe, K., Misaka, T., & Ishiguro, M. (2012). Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds. *PLoS One, 7*(4), Article e35380.

Muraoka, T., Ishihara, K., Oyamada, C., Kunitake, H., Hirayama, I., & Kimura, T. (2008). Fermentation properties of low-quality red alga Susabinori Porphyra yezoensis by intestinal bacteria. *Bioscience Biotechnology and Biochemistry, 72*(7), 1731–1739.

Nagasawa, T., Ishimaru, K., Higashiyama, S., Hama, Y., & Mitsutake, S. (2020). Teadenol A in microbial fermented tea acts as a novel ligand on GPR120 to increase GLP-1 secretion. *Food & Function, 11*(12), 10534–10541.

Nagasawa, T., Nakamichi, H., Hama, Y., Higashiyama, S., Igarashi, Y., & Mitsutake, S. (2018). Phytosphingosine is a novel activator of GPR120. *Journal of Biochemistry, 164*(1), 27–32.

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell, 106*(3), 381–390.

Noda, H., H, A., Abo, K., & Horiguchi, Y. (1981). Sugars, organic acid, and minerals of 'Nori', the dried laver Porphyra spp. *Bulletin of the Japanese Society of Scientific Flsheries, 47*(1), 57–62.

Nuemket, N., Yasui, N., Kusakabe, Y., Nomura, Y., Atsumi, N., Akiyama, S., Nango, E., Kato, Y., Kaneko, M. K., Takagi, J., et al. (2017). Structural basis for perception of diverse chemical substances by T1r taste receptors. *Nature Communications, 8*(1), Article 15530.

Putman, E. W., & Hassid, W. Z. (1954). Structure of galactosylglycerol from irideae laminarioides. *Journal of the American Chemical Society, 76*(8), 2221–2223.

Rindi, G., Grant, S. G., Yiangou, Y., Ghatei, M. A., Bloom, S. R., Bautch, V. L., Solcia, E., & Polak, J. M. (1990). Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. *American Journal Of Pathology, 136*(6), 1349–1363.

Simon-Colin, C., Kervarec, N., Pichon, R., & Deslandes, E. (2002). Complete 1H and 13C spectral assignment of floridoside. *Carbohydrate Research, 337*(3), 279–280.

Simon, B. R., Parlee, S. D., Learman, B. S., Mori, H., Scheller, E. L., Cawthorn, W. P., … MacDougald, O. A. (2013). Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. *Journal of Biological Chemistry, 288*(45), 32475–32489.

Stone, L. M., Barrows, J., Finger, T. E., & Kinnamon, S. C. (2007). Expression of T1Rs and gustducin in palatal taste buds of mice. *Chemical Senses, 32*(3), 255–262.

Thomsen, R., & Ghristensen, M. H. (2006). MolDock: A new technique for high-accuracy molecular docking. *Journal of Medical Chemistry, 49*(11), 3315–3321.

Winnig, M., Bufe, B., Kratochwil, N. A., Slack, J. P., & Meyerhof, W. (2007). The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor. *BMC Structural Biology, 7*(1), 66.