BioMol Concepts, Vol. 2 (2011), pp. 327–332 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515.BMC.2011.021

Short Conceptual Overview

The discovery and mechanism of sweet taste enhancers

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Abstract

Excess sugar intake posts several health problems. Artificial sweeteners have been used for years to reduce dietary sugar content, but they are not ideal substitutes for sugar owing to their off-taste. A new strategy focused on allosteric modulation of the sweet taste receptor led to identification of sweet taste 'enhancers' for the first time. The enhancer molecules do not taste sweet, but greatly potentiate the sweet taste of sucrose and sucralose selectively. Following a similar mechanism as the natural umami taste enhancers, the sweet enhancer molecules cooperatively bind with the sweeteners to the Venus flytrap domain of the human sweet taste receptor and stabilize the active conformation. Now that the approach has proven successful, enhancers for other sweeteners and details of the molecular mechanism for the enhancement are being actively pursued.

Keywords: G-protein-coupled receptor; positive allosteric modulator; sweet taste enhancer; sweet taste receptor; umami taste receptor; Venus flytrap domain.

Introduction

Mammals use their sense of taste to guide their feeding behavior (1). Among the five basic taste qualities, sweet, umami and salty taste elicit primarily attractive responses and promote consumption of nutritive food, whereas bitter and sour taste trigger repulsive reactions and lead to rejection of potentially harmful substances. Sweet taste has evolved to detect and promote the consumption of carbohydrate, the common source of energy for living organisms. In general, the monosaccharides and disaccharides, commonly referred to as sugars, are the primary natural sweet taste stimuli.

Throughout human evolution, the availability of food has in general been limited. Overnutrition is only a recent phenomenon in parts of the human world. In the late 19th century, the annual average sugar consumption in the US was $<$2.5 kg per person. At the beginning of the 21st century, the average amount was a staggering 68 kg (2). Excessive calories from sugar can lead to obesity and increased risk of diabetes and several other metabolic syndromes (2). Overnutrition has developed into a major health concern in a very short period of time, probably because evolution has not equipped the human body with an effective mechanism to deal with excess nutrients.

The food and beverage industry has been increasingly replacing sugar with substitutes in a range of products traditionally containing sugar, to address not only the health concern but also to reduce the cost of goods. Several sugar substitutes have been developed, the majority of which are artificial sweeteners. In the US, six intensely sweet sugar substitutes including stevia, aspartame, sucralose, neotame, acesulfame potassium, and saccharin, have been approved for use. However, none of those sweeteners can reproduce the real sugar taste. Their common undesirable characteristics include bitter/metallic off-taste, slow sweetness onset, and sweetness linger.

Recently, a new approach to reduce dietary sugar content using allosteric modulators has proven successful (3). Sweet taste 'enhancers', which do not taste sweet on their own, can greatly potentiate the sweet taste of sugar allowing lowered sugar content without reduction in sweet taste (3). In this article, we review the discovery, taste effect, and molecular mechanisms of this class of enhancer molecules.

The sweet taste receptor

Taste is mediated by a group of specialized chemosensory cells known as taste receptor cells (TRCs). Clusters of 50–100 TRCs form a taste bud (Figure 1), an onion-shaped assembly distributed on the surface of the tongue and soft palate. The cluster of elongated TRCs project microvillae to the apical surface and form the 'taste pore' at the top of the taste bud. Taste receptor proteins are concentrated at the taste pore and exposed to the oral cavity. This is where tastant molecules come in contact with the receptor proteins and where taste detection is initiated. When they bind to and activate the taste receptors, the tastant molecules can trigger taste-specific signal transduction pathways and lead to activation of the TRCs. The signal is relayed to the brain through either the chorda tympani or the glossopharyngeal nerves.

The molecular identities of taste receptors only started to be elucidated over the past 12 years (4–15). It is now known that mammalian sweet taste receptor is a heteromeric complex of two proteins, T1R2 and T1R3 (12, 15). There are three genes in the T1R family, and they encode two taste receptors: T1R2/T1R3 for sweet taste, and T1R1/T1R3 for umami taste (14, 15). The T1Rs are class C G-protein-coupled receptors (GPCRs). Other renowned members of this class of GPCRs include the metabotropic glutamate receptors (mGluRs), γ-aminobutyric acid receptor B, and the calcium

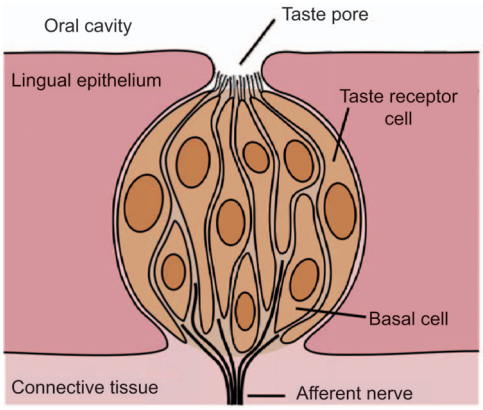

Figure 1 Schematic drawing of a taste bud.
Source: http://commons.wikimedia.org/wiki/File:Taste_bud.svg.

sensing receptor. The defining motif of this class of GPCRs is the extracellular Venus flytrap (VFT) domain (Figure 2), which is their natural ligand binding domain. The VFT domain is so-named because of its structural resemblance to the leaves of the Venus flytrap plant, a carnivorous plant that catches animal prey. The crystal structures of mGluR VFT domains have been solved (16, 17). The domain is composed of two globular subdomains connected by a three-stranded flexible hinge. The bi-lobed architecture can form an 'open' or 'closed' conformation (16, 17). The closed conformation of the VFT domain is stabilized by its ligand, analogous to the closure of the Venus flytrap leaves after trapping its prey.

Identification of sweet taste enhancers

After identification of the human sweet taste receptor, a new approach of reducing dietary sugar intake became possible. Recently in the pharmaceutical industry, allosteric modulators of GPCRs have emerged as attractive alternatives to orthosteric agonists and antagonists for development of novel therapeutic agents (18, 19). This approach can also be applied to taste receptors. A positive allosteric modulator (PAM) for the sweet taste receptor would potentiate the receptor activity, make sugar taste sweeter and therefore allow a reduced sugar content in foods and beverages. In support of this concept, many PAMs have been identified for several other class C GPCRs. More importantly, the closely related umami taste receptor (20) has naturally occurring PAMs in purinergic ribonucleotides, such as inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP).

Using our proprietary human sweet receptor high-throughput screening technology, we identified the first proof-of-concept sweet taste enhancer, SE-1 (3). Interestingly, SE-1 is not a 'general' sweet enhancer but is highly selective for sucralose. Among the analogs of SE-1, a more potent sucralose enhancer, SE-2, and more importantly a sucrose enhancer, SE-3, were quickly identified (Figure 3).

The activities of SE-2 and SE-3 were validated in human taste tests (3). Although neither compound elicits sweet taste when tasted alone, SE-2 can make a 100 ppm sucralose solution taste as sweet as 600 ppm, and SE-3 can make a 6% sucrose solution taste like 8–9%. Since the discovery of these proof-of-concept molecules, significantly improved enhancers, which allow approximately 50% sucrose reduction in product prototypes, have been identified for sucrose through screening and optimization guided by receptor assay and taste tests. Distinct from artificial sweeteners, the optimized enhancers do not introduce any off-taste or slow onset/lingering effects.

Previously, sweet taste synergy was reported among different sweeteners (21, 22) and several molecules have been proposed, or even marketed, as sweet taste enhancers. However, they are all sweeteners used near their sweet taste threshold level. Their marginal taste effects are not enhancement, but additivity, owing to their inherent sweet taste (23). We can use sucrose for an analogy. A solution of 1% sucrose is barely sweet; adding an extra 1% of sucrose to a 6% solution would make it taste sweeter, but that does not qualify 1% sucrose as a real sweet enhancer. The validity of enhancement by previously reported sweet enhancers has not been established, and they are not in the same category as the SE series of molecules.

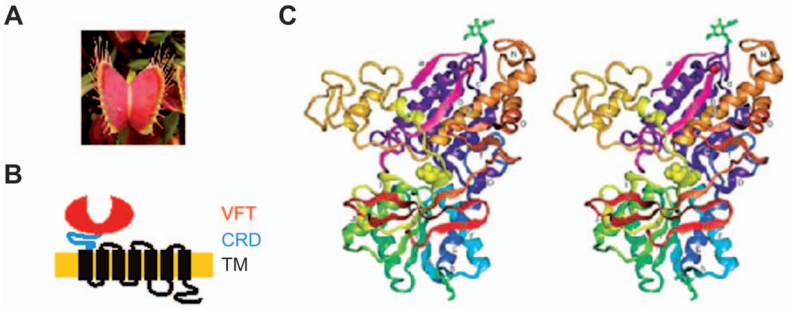

Figure 2 The VFT domain.
(A) Picture of a leaf of Venus flytrap (*Dionaea muscipula*) (http://en.wikipedia.org/wiki/Venus_Flytrap). (B) Schematics of a T1R. Red: VFT domain; cyan: CRD domain; black: TM domain. (C) A stereoview of the mGluR1 VFT domain in a closed conformation. Reproduced with permission from Ref. (16).

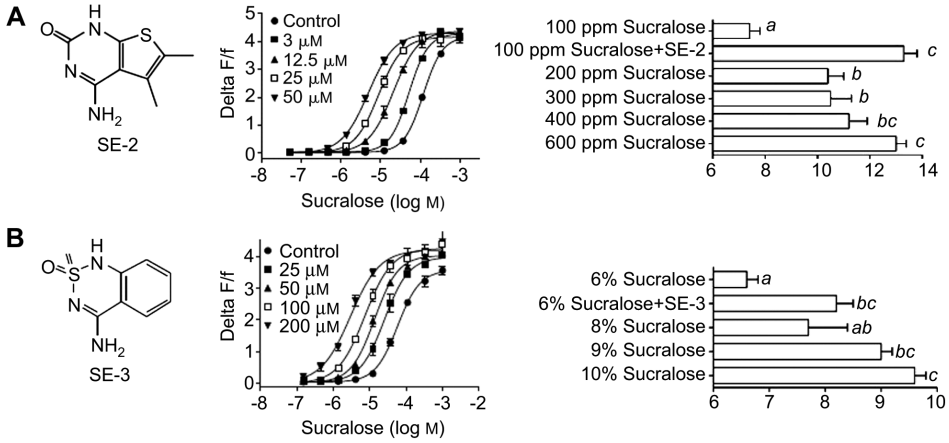

Figure 3 The structures, activities in the sweet receptor assay, and taste effects of SE-2 (A) and SE-3 (B).

The molecular mechanism of umami taste enhancers

To understand the molecular mechanisms of the sweet taste enhancers, it is worthwhile to take a closer look at the related umami taste receptor, where enhancement occurs naturally. The natural umami tastants include L-glutamate, L-aspartate, and purinic ribonucleotides, such as IMP and GMP. A unique feature of synergy (24) is well documented in umami taste research: the mixture of an umami tasting amino acid with a purinic ribonucleotide gives much stronger umami taste than the sum of either class of umami tastant alone. Submillimolar concentrations of IMP or GMP, which elicit no umami taste on their own, can greatly potentiate the umami taste of glutamate or aspartate. IMP and GMP are umami taste enhancers, the only known natural enhancers of any GPCRs.

The principal mechanism of umami synergy was recently elucidated using a combination of chimeric receptors, mutagenesis, and molecular modeling approaches (20). Although both glutamate and IMP require the T1R1 VFT domain for their activities, they occupy different parts of the binding pocket. Four residues near the hinge region were found to be crucial for glutamate activity, whereas another four residues near the lips were crucial for IMP activity. A homology model (Figure 4) of the T1R1 VFT based on the crystal structure of metabotropic glutamate receptors was proposed to explain the synergy: glutamate binds close to the hinge region of the VFT domain and induces the closure of the lobes, which activates the receptor. IMP binds close to the opening of the VFT domain and coordinates the positively charged residues from both sides of the bi-lobed structure, thereby stabilizing the closed conformation and enhancing the activity of the receptor. There are probably direct interactions between glutamate and IMP, which could increase the binding affinity of both ligands. The cooperative binding model for umami synergy represents a novel mechanism of GPCR modulation. Many synthetic allosteric modulators for class C GPCRs have been developed in recent years. However, all of them bind to the transmembrane domain of their

A

	Activities		
hT1 R1	Glutamate	IMP	S807
WT	+	+	+
H71A	+	-	+
R277A	+	-	+
S306A	+	-	+
H308A	+	-	+
S172A	-	+	+
D192A	-	+	+
Y220A	-	+	+
E301A	-	+	+

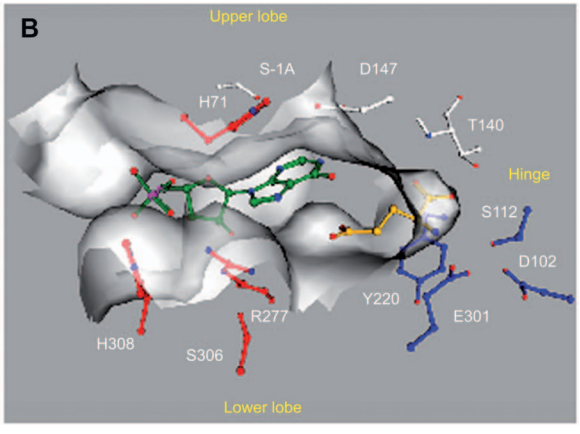

Figure 4 Crucial residues for glutamate and IMP recognition (A) and schematics of the molecular model to explain the synergy between glutamate and IMP (B).
The crucial residues for IMP enhancement activity are in red and those for glutamate activity are in blue. S807 is a control umami agonist that targets the transmembrane domain of T1R1.

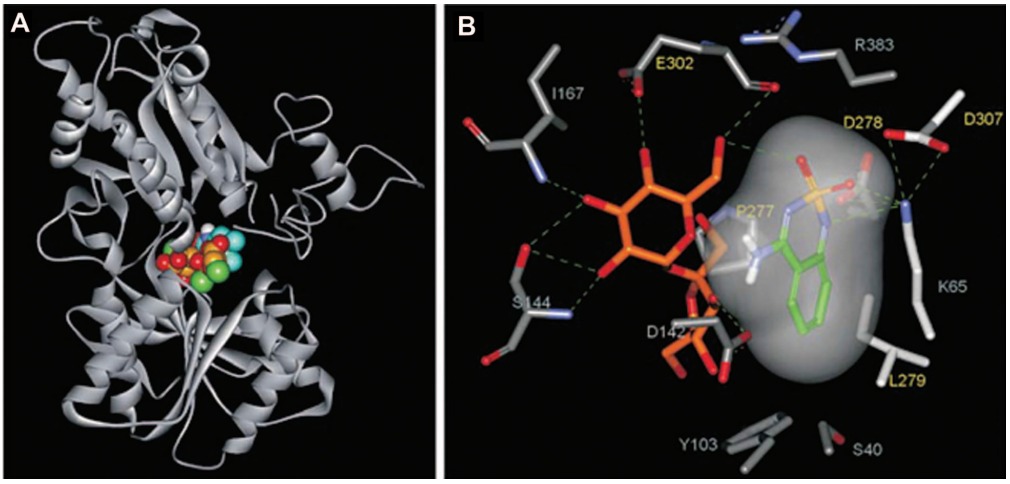

Figure 5 A molecular model of the T1R2 VFT domain.
(A) The T1R2 VFT domain in a closed conformation with bound sucralose (carbon atoms in gold, oxygen in red, and chlorine in green) and SE-2 (carbon atoms in cyan). (B) A close view of the ligand binding pocket looking down from above the upper lobe with sucrose and SE-3 bound. Lower lobe residues are labeled in yellow letters and upper lobe residues in gray letters. Sucrose is shown in gold; SE-3 is in green and is encased in a gray surface. The three crucial residues for enhancer activities (K65, L279, and D307) are in white, and the seven crucial residues for sucrose/sucralose activities (S40, Y103, D142, D278, E302, P277, and R383) are in gray.

target GPCRs (18, 19), apparently working through mechanisms different from the umami taste enhancers.

The molecular mechanism of sweet taste enhancers

Multiple ligand binding sites have been identified on the sweet taste receptor. In addition to the T1R2 VFT domain where the majority of sweeteners bind (20), the T1R3 transmembrane domain is responsible for interacting with some sweeteners (25–27), such as cyclamate, and the sweet taste inhibitor lactisole (25, 28, 29), whereas the T1R3 cysteine rich domain (CRD) is crucial for the activity of the sweet protein Brazzine (30). Many new ligands have been identified at Senomyx by screening the human sweet and umami taste receptors. The majority of those molecules interact with the T1R transmembrane domains. In contrast to what had been observed in other class C GPCRs, no enhancer was identified among the molecules that target the transmembrane domains. They all turned out to be agonists.

After the sweet enhancer molecules were identified, it became clear that enhancement of the sweet taste receptor follows a mechanism similar to that of the umami taste receptor (31). Using both human rat and sweet umami chimeric receptors, we mapped the SE-2 and SE-3 binding site to the T1R2 VFT domain. Mutagenesis studies showed a similar binding pattern as observed for enhancers of the umami receptor. A set of seven residues was shown to be crucial for sucrose/sucralose activities. Mutation of each one of these residues affected both sucrose and sucralose activities but not the enhancer activities. Another three residues were shown to be more important for the enhancer activities. Mutations of this set of residues abolished the enhancer activities while leaving the sucrose/sucralose activities largely intact.

Homology models (Figure 5) revealed a large cavity in the upper lobe of the T1R2 VFT domain, which could explain the diverse chemical structures of sweeteners that can fit in the binding pocket. According to this model, sucrose/sucralose interacts with the backbone nitrogen of I167 and S144 and to the hydroxyl of S144. Both residues are close to the hinge region. All hydroxyl groups of sucrose and sucralose made hydrogen bonds with adjacent hydrophilic residues, such as D142 and E302, whereas the chlorines of sucralose found hydrophobic contacts with residues Y103 and P277. The enhancers bind adjacent to the sweeteners. In the closed conformation with sucrose and SE-3 bound, four residues (K65, L279, D278, and D307) surround and interact with the enhancer. Through interactions with residues from both upper and lower lobes, the sweet enhancers stabilize the closed conformation of the VFT domain and thereby enhance receptor activity. The fact that these sweet enhancers are selective for sucrose/sucralose suggests that there are likely interactions between the enhancers and the sweeteners. In the model, sucrose and SE-3 form direct contact through a hydrogen bond. The binding mode of sucralose and SE-2 follows a similar pattern, with sucralose and SE-2 in direct contact, and both form extensive hydrogen bonding patterns to residues in the active site.

The closure of the VFT domain brings the upper and lower lobes closer together and facilitates interlobe interactions (15). In our model, extensive electrostatic and hydrophobic interactions between the two lobes were observed in the closed conformation. The potential electrostatic pincer residues include R383 and K65 of the upper lobe and D278 and D307 of the lower lobe. The potential hydrophobic pincer residues include A43, V64, I67, Y103, and K65 of the upper

lobe, and P277, L279, and V309 of the lower lobe. K65 appears to be involved in both electrostatic and hydrophobic interactions. Even though these residues are not in direct contact with sucrose or sucralose in our model, mutations of some of these residues still resulted in a diminished response to the sweeteners. This is probably due to the crucial role of these residues in stabilizing the closed conformation. The most convincing interlobe interaction was between K65 and D278, demonstrated by mutagenesis studies. Reversing the charge on either residue individually (K65D or D278K) abolished the response of the receptor to sweeteners. However, the responses were rescued by the double mutant (K65D/D278K) where the two residues switch position. This observation indicates that the electrostatic interactions between K65 and D278 are crucial for sweet taste receptor activity.

Many large sweeteners, such as stevioside, interact with the T1R2 VFT domain with much higher affinity than sucrose. The model suggests that these sweeteners occupy both the sucrose and enhancer binding sites and this notion is supported by mutagenesis data.

Concluding remarks

After years of effort, verified sweet taste enhancers have finally been identified. The enhancers do not introduce any off-taste. A potentiated real sugar taste is achieved for the first time with the sucrose enhancer. This class of enhancer molecules follows a similar mechanism as the natural umami enhancers by binding the VFT domain of T1R2 cooperatively with the sweeteners to stabilize the active conformation. The enhancers are selective for sucrose/sucralose, which is determined by the nature of the cooperative binding. The search for enhancers of other sweeteners is in progress. Molecular modeling successfully predicted the electrostatic interaction between K65 and D278 located on the opposite lobes of the VFT domain. However, the model could only provide a very rough picture of how the enhancers and sweeteners fit in the VFT binding pocket. As stated by George Box (32): ‘Essentially, all models are wrong, but some are useful.’ Although we have confidence in the general nature of cooperative binding between enhancers and sweeteners, the details of the interactions await to be revealed by future structural biology studies.

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Received March 3, 2011; accepted May 11, 2011