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Review

Bitter peptides and bitter taste receptors

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Abstract. Bitter peptides are a structurally diverse group of oligopeptides often generated in fermented, aged, and hydrolyzed food products that make them unfavorable for consumption. Humans perceive bitterness by a repertoire of 25 human bitter receptors, termed T2Rs. Knowledge of the structural features of bitter receptors and of the factors that stimulate bitter receptors will aid in understanding the mechanism responsible for bitter taste perception. This article reviews the current knowledge regarding structural features of bitter peptides and bitter taste receptors.

Keywords. Bitter peptide, casein hydrolysate, bitter receptor, T2Rs, structure.

Introduction

All animals, including humans, need to ingest organic compounds as nutrients, but ingestion of toxic substances can be life-threatening. Most animals are instinctively averse to bitter-tasting substances, which serves to protect against ingesting bitter-tasting toxic substances. For humans, bitter taste aversion is also innate. However, humans prefer bitterness to some extent in certain foodstuffs, for example, beer, coffee, and tea, perhaps because tolerance to bitter tasting foodstuffs with positive pharmacological effects may change with age. It is widely known that bitterness is frequently generated during the aging process in fermented products and during the enzymatic processes that produce protein hydrolysates.

In these cases, the acceptability of bitterness depends on its intensity – on the nature and amount of the short-chain peptides formed from the breakdown of proteins. But not all peptides are bitter; for example, aspartame (L-aspartyl-L-phenylalanine methyl ester) [1] is intensely sweet, and several acidic oligopeptides isolated from fish protein hydrolysate have a flavor-enhancing quality resembling that of monosodium glutamate [2]. Octapeptide, which is a constituent of papain-treated beef, has an appealing taste [3 – 7] but is not strong enough to be the principal taste constituent of foodstuffs, in which many peptides contribute to palatability [8, 9].

Although savory and palatable tastes develop when protein is hydrolyzed into small peptides and amino acids, partial degradation of protein due to abnormal proteolysis causes excessive bitterness and decreases the sensory quality of products. Therefore, bitter peptides have been extensively studied with the aim of improving the sensory properties of foodstuffs and beverages. Many bitter peptides have been isolated and identified from protein-rich foods and protein hydrolysates, and the structural aspects related to bitter taste have been partially established from studies on the structure-activity relationships of peptides.

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On the other hand, studies on bitter taste perception have been conducted independent of research on bitter peptides. When the "bitter unit" was first proposed two decades ago as the activating site on bitter peptides [10], the bitter taste receptors that are stimulated by these bitter peptides – currently known as T2Rs – had not yet been identified. Recent molecular studies have revealed that the T2Rs expressed in taste receptor cells function as bitter taste receptors. The peripheral molecular mechanisms transducing bitter taste are described in many reviews [11–17]. This review focuses on our current knowledge of the structural relationships between bitter peptides and human bitter receptors.

Bitter peptides in protein hydrolysates

Many substances are recognized as being principally responsible for taste, including some peptides. A wide variety of peptides derived from proteins have been found in various foodstuffs and fermentation products, such as miso (salted and fermented soybean paste) [18], soy sauce [19, 20], fish sauce [21], natto (fermented soybeans) [22], katsuobushi (dried fish flakes) [23], cheese [24–27], and sake [28, 29]. Hydrolysis of proteins by proteolytic enzymes is also usually accompanied by the formation of a bitter taste [30–33]. Bitter peptides are produced during food processing: They occur in protease-treated proteins as well as in fermented and aged foods.

One of the earliest reports addressing bitterness in food processing is by Murray and Baker [34]. In their 1952 study, they found that hydrolysates made from gelatin and casein develop a bitter and unpleasant taste, and that the bitter taste of a casein hydrolysate can be decreased by treatment with activated carbon – a strongly bitter polypeptide-rich fraction can be eluted from the spent carbon. These preliminary observations indicated that bitter taste is due to peptides, rather than free amino acids, and that peptides can be adsorbed to a hydrophobic adsorbent. Shortly afterward, Raadsvelt [35] announced that the bitter taste of cheese is primarily due to a peptide. Because of its industrial importance, the development of bitter taste in cheese during ripening has received particular attention. Apparently, casein produces the most bitter hydrolysates, and digestion of proteins by proteolytic enzymes is always accompanied by the formation of bitter substances [32]. The taste properties of protein hydrolysates vary depending on the type of protein and enzyme used [9].

Fermented milk products are avoided by many people because of their considerable bitterness, which is due to peptides. Interestingly, whereas adults and older children avoid the bitterness of peptides, infants four months old and younger accept them without difficulty [36]. It is hypothesized that there is an early period of sensitivity during which the hedonic value of bitterness of milk protein hydrolysates, and likely the flavors of other foods and beverages, is established [37–39].

Rejection of casein hydrolysates is also observed in animals. Hydrolyzed casein was recently shown to significantly reduce browsing by deer, even when alternative food choices were minimized [40]. Hydrolyzed gelatin is also a prospective herbivore repellent. The effectiveness of hydrolyzed casein and gelatin is species dependent; these substances have been shown to be effective at repelling captive rabbits, pocket gophers, voles, and mountain beavers. Formulations of 8–12% hydrolyzed casein have been shown to be as effective as commercially available products at repelling deer from damaging western red cedar seedlings [40–42]. Casein hydrolysate is also avoided by mice, which may be a useful model for understanding the mechanisms of hydrolyzed casein rejection. The avoidance of protein hydrolysates by these animals is not nutritionally driven, but is actually the result of a factor within the protein that these animals find unpalatable [43]. In fact, animals should choose hydrolyzed casein-containing foods over casein-containing foods when nutritionally compromised, due to its shorter gastrointestinal transit time and increased rate of amino acid adsorption compared to intact casein [44].

Bitterness also develops from soy protein products, which is of great concern because of the worldwide demand for edible plant protein sources and because soybean protein and soybean-derived peptides can play an important role in physiology, particularly the prevention of chronic diseases (reviewed in [45]). Kim et al. [46] observed a reduction in molecular size and the formation of bitterness during the tryptic hydrolysis of soybean 11S glycinin; based on gel permeation HPLC analysis, peptic fractions primarily of 0.36–2.10 kDa were responsible for the bitterness. Arai et al. [47] found that each of the several bitter peptides isolated from peptic hydrolysates of soy protein had a leucine residue at the C-terminus; applying carboxypeptidase, which degrades the C-terminal structures, markedly decreased their bitter flavor. Natto, a traditional Japanese food made from soybeans fermented with bacterial proteinases, often has a bitter taste due to inadequate fermentation. A partial amino acid sequence of the peptide responsible for its bitterness showed that the N- and C-termini of the peptide were both leucine [47].

Recent research has shown that some food-borne peptides have various health-related physiological

activities, including antihypertensive (angiotensin I-converting enzyme [ACE] inhibition), opioid, immunomodulating, antioxidative, antimicrobial, antithrombotic, antiamnestic, and hypocholesterolemic activities (for reviews, see [48–53]). Many proteins from animal and plant sources contain potential bioactive sequences, especially milk proteins, which are the primary source of a range of biologically active peptides (reviewed in [54]).

Peptides that inhibit ACE, which regulates peripheral blood pressure, have been investigated extensively. Many ACE inhibitory peptides have been isolated and identified from various fermented foods [55]. Many bitter dipeptides have ACE-inhibitory activity [56]. The structures of biologically active sequences can be ascertained via *in vitro* enzymatic and/or *in vivo* gastrointestinal digestion of the appropriate precursor proteins.

Structure-bitterness relationships in peptides

After reviewing the amino acid composition of bitter peptides, Ney [57] postulated that a correlation exists between the hydrophobicity of peptides and their bitter taste. He calculated the Q value – the average amount of free energy needed for the transfer of amino acid chains from ethanol to water – and found that all bitter peptides have a Q value greater than 1400 kcal/mole, whereas all nonbitter peptides have a Q value less than 1300 kcal/mole. The empirical correlation between the presence or absence of bitterness and average hydrophobicity is called the Q rule. This rule is empirically based and reflects the assumption that a hydrophobic interaction is essential for the sensation of bitter taste, but that the sequences of the amino acids in the peptides have no influence on bitter taste. Guigoz and Solms [58] compared the Q value data from Tanford [59] for 61 bitter-tasting peptides from many protein-rich foods and for 145 synthetic bitter peptides. Their data supported the notion that the average hydrophobicity of peptides should be useful in assessments of the relationships between amino acid composition and the bitter taste of peptides and protein hydrolysates. However, the bitterness of a peptide cannot be predicted simply on the basis of its Q value, and inconsistency has been reported between some peptides' bitter activities and their Q values [60].

Structure-activity relationships for sweet taste molecules have been extensively studied. Shallenberger [61–63] proposed a theory in which sweet-tasting compounds have structural units in common: An AH unit (hydrogen donor) and a B unit (hydrogen acceptor), where H^+ is an acidic proton and B is an electronegative atom or center. The distance between the proton and the B unit is about 3 Å, and a variety of functional groups satisfy these requirements.

Attempts have also been made to elucidate structure-activity relationships for bitter-taste molecules. Many investigators have identified bitter peptides from natural sources [24, 31, 32, 64], and the structural requirements of peptides for bitterness have been proposed using synthetic peptides [10, 65–82]. Natural and synthetic bitter peptides related to casein peptide are listed in a review by Roudot-Algaron [83]. Matoba et al. [31] purified three kinds of peptides from the tryptic hydrolysate of casein that were strongly bitter in a 0.1% solution. The primary structures of these peptides were proposed to be Gly-Pro-Phe-Pro-Val-Ile, Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Val-Lys, and Phe-Ala-Leu-Pro-Glu-Tyr-Leu-Lys. Because phenylalanine was common to these bitter peptides, it was predicted to be a functional residue in the development of a bitter taste. L-Phenylalanine has a slightly bitter taste, but its bitterness is strongly enhanced when its amino and carboxyl groups are blocked by acetyl and ethoxy groups, respectively. This suggests that amino and carboxy groups may contribute to the weakening of the bitterness of phenylalanine; that is, its bitterness is intensified when those groups are blocked by the formation of peptide bonds.

Matoba and Hata [84] synthesized various peptides and derivatives of peptides and amino acids to systematically investigate their structure-bitterness relationships. Glycyl peptides containing amino acids with hydrophobic side chains, such as Gly-Leu, Val-Gly, and Gly-Phe-Gly, were all bitter. However, other glycyl peptides, such as Gly-Gly, Gly-Ala, and Gly-Glu, were not bitter. The bitterness of hydrophobic amino acids intensified when they were introduced into peptide chains, and was stronger when both amino and carboxy groups were blocked than when only one side was blocked. Furthermore, the role of amino and carboxyl groups in the bitterness of peptides was investigated using acetylated and esterified amino acids as a simple peptide model [84]. Hydrophobic amino acids were bitterer when amino and/or carboxyl groups were blocked by acetylation and esterification, and acetyl amino acid methyl esters such as Ac-Leu-OMe were bitterer than acetyl amino acids and methyl esters with only one group blocked. Peptides were also bitterer than the mixture of amino acids constituting the respective peptide. For example, free forms of leucine and phenylalanine were bitter, with thresholds of 15–20 mM, but Leu-Leu and Leu-Phe were 10 times more bitter. The peptides whose amino and carboxyl groups are blocked by acetylation and esterification are also 10 times more bitter than

their corresponding peptides, which also indicates that bitterness is intensified when both amino and carboxyl groups of the peptides are blocked by acetylation and esterification.

Bitter peptides of various chain lengths have no specific primary structures, but they have a high content of amino acids and a hydrophobic side chain in common. In aqueous solution, proteins have hydrophilic or polar groups on their outer surface, with the hydrophobic groups packed inside. Enzymatic digestion of proteins exposes the peptide moieties, which contain large amounts of hydrophobic amino acids – these give a sensation of bitterness when they come in contact with taste receptors.

It is widely accepted that a hydrophobic amino acid residue is essential for the manifestation of bitterness. However, the overall hydrophobicity of a peptide does not directly correlate with its bitterness. The structure-bitterness relationship has been systematically studied using more than 200 synthetic peptides [10, 65–82] based on the structure of Arg-Gly-Pro-Pro-Phe-Ile-Val (threshold bitterness value [TV] = 0.05 mM), which was isolated from casein hydrolysates by Minamiura et al. [32]. Ishibashi et al. [10] compared the bitterness of valine- or leucine-containing peptides with that of norvaline- or norleucine-containing peptides, respectively, and found that the linear side chain of norvaline and norleucine effected a more intense bitterness than did the branched chain of their analogues. The intensity of bitterness was most significantly affected by the number of carbons or the hydrophobicity of the side chain of the amino acid. For the bitter taste to be generated, the side-chain skeleton needed to consist of at least three carbons.

Ishibashi et al. [82] reported that the bitter taste of Arg-Gly-Pro-Pro-Phe-Ile-Val is attributed to the spatial structure of the L-proline residue. From sensory testing with Arg-Pro (TV = 0.8 mM) and its analogues, such as Arg-Arg (TV = 8 mM), Arg-Gly (TV = 10 mM), Gly-Arg (TV = 100 mM), Pro-Arg (TV = 3 mM), Arg-Gly-Pro (TV = 13 mM), Arg-Pro-Gly (TV = 0.8 mM), Gly-Arg-Pro (TV = 0.8 mM), and so on, they concluded that the most significant role of the proline residue in peptide bitterness depends on the conformational alteration of the peptide molecule (i.e., the folding of the peptide skeleton due to the imino ring of the molecule). Furthermore, they proposed that the hydrophobic imino ring functioned as a binding unit (BU) for the bitter taste receptor, and that the adjacent guanidino group in the arginine residue played a role as the stimulating unit (SU). Bitter taste was apparent only when BU and SU coexisted. The hydrophobic group of the peptide offers a binding site for the bitter taste receptor.

The distance between the two bitter-taste-determinant sites was measured by devising molecular models of three cyclic dipeptides, cyclo(-Arg-Phe), cyclo(-Arg-Pro-), and cyclo(-His-Phe-) [10], since guanidino groups in arginine, phenyl groups in phenylalanine, imidazole groups in histidine, and diketopiperazine and imino rings in proline had already been recognized as bitter taste determinant sites in bitter peptides. The average distance between the two sites BU and SU was estimated to be 4.1 Å. The BU that determines the bitterness of the peptide is the bulky hydrophobic group, which is composed of a skeleton with at least three carbons. The imino ring or the diketopiperazine ring in proline also plays the role of this hydrophobic group. A bulky basic group, including an α-amino group, serves as the SU site. A hydrophobic group also serves as the SU site for bitter taste.

From this view, a mode for binding of bitter peptides with bitter receptors was proposed (Fig. 1). To further explain the mechanism responsible for the intensity of bitterness, Tamura et al. [84] proposed that the receptor recognizes the hydrophobicity of the peptide, and that the intensity of the bitterness is determined at a hydrophobicity recognition zone located on the wall of a pocket of the receptor when the BU and SU are located at the bottom of the receptor. Bitter peptides are composed of fewer than eight amino acids, and the bitterness of the peptides increases as the number of amino acids increases. Peptides composed of eight or more amino acids do not differ in bitter potency and form spherical shapes instead of a helix conformation. From the sensory analyses of thousands of bitter peptides, the size of the "pocket" of the bitter receptor is estimated to be approximately 15 Å.

Bitter taste perception

Mammals can perceive five basic tastes (sweet, sour, bitter, salty, and umami) via taste receptor cells (TRCs), which are assembled into taste buds. Taste buds are distributed across different papillae of the tongue. For some time, it has generally been accepted, despite a lack of experimental evidence, that a "tongue map" exists in which bitterness is perceived at the very back of the tongue and sweetness is perceived at the front of the tongue. However, recent molecular and functional data have revealed that responsiveness to five basic tastes is distributed in all areas of the tongue.

Sweet, bitter, sour, salty, and umami tastants are recognized by different TRCs expressing specialized receptors [16]. Competing models explain how these TRCs signal taste sensation to the brain. In one model,

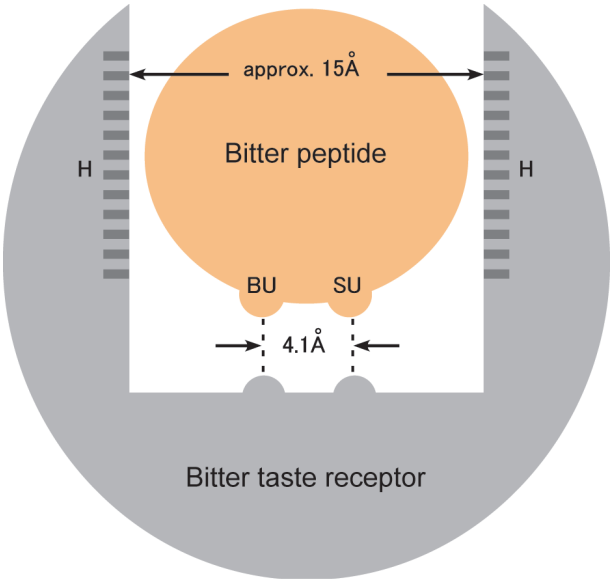

Figure 1. Model for binding of bitter peptides to bitter receptors proposed by Ishibashi et al. [10] and Tamura et al. [85]. Bitter peptides possess two determinant sites, the binding unit (BU) and the stimulating unit (SU). The bulky hydrophobic group functions as the BU, the primary site that determines the bitterness of the peptide. The bulky basic group, including an α-amino group or hydrophobic group, functions as the SU, a secondary site that also contributes to bitter taste. The intensity of bitterness is hypothesized to depend on the features of both the BU and the SU and on the distance between them. The optimal distance is estimated to be 4.1 Å. The size of the pocket of the proposed bitter receptor is around 15 Å, which could allow contact with a peptide of fewer than eight amino acids. Once in contact, the bitter receptor detects the hydrophobicity of the peptide by the hydrophobicity recognition zone (H), which is probably located on the wall of the pocket and stimulates the various bitter potencies.

called the labeled-line model, coding at the periphery is independent for each taste modality. In an alternative model, TRCs are broadly tuned across taste modalities, and tastant recognition results from the combined responsiveness of various TRCs that express different types of taste receptors. Recent molecular and functional studies in mice have shown that different TRCs define the different taste modalities and that activation of a single type of TRC is sufficient to encode taste quality, which strongly supports the labeled-line model.

Bitter taste receptors

Recent molecular studies have revealed that specific receptors expressed on TRCs mediate the five basic tastes. The first taste receptor candidates were the receptors T1R1 and T1R2 (formerly known as TR1 and TR2, respectively), which were cloned and identified as members of class C G-protein–coupled receptor (GPCR) subfamily. These taste receptors were found to be specifically localized to the taste pore [86]. Another member of the T1R receptor family, the candidate taste receptor gene *T1r3*, was identified at or near the mouse *Sac* locus – a genetic locus that controls the detection of certain sweet tastants. The observation that T1R3 expression is detected in the same TRCs as is T1R2 by *in situ* hybridization raised the possibility that two receptors function as heterodimers [87]. In a heterologous expression system, T1R2 or T1R3 alone did not respond to sweet stimuli, suggesting that the putative T1R2/T1R3 heterodimer forms a functional sweet receptor. However, another experiment indicated that although T1R3 alone cannot respond to artificial sweeteners or low concentrations of natural sugars, it can respond to high concentrations of natural sugars (> 300 mM) [88]. The T1R2/T1R3 heterodimer has been shown to recognize sweet-tasting molecules as diverse as sucrose, saccharin, dulcin, and acesulfame-K [89].

TRCs detect bitter stimuli via the T2R GPCRs, triggering a G-protein–mediated signaling cascade. Several G-protein α-subunits have been identified in TRCs, including Gαi2, Gαi3, Gα14, Gα15, Gαq, Gαs, α-transducin, and the α-transducin-like subunit α-gustducin [90–92]. Nearly all T2Rs are localized in α-gustducin–expressing TRCs, but not all α-gustducin–expressing TRCs possess bitter receptors – other α-gustducin-expressing cells possess sweet or umami receptors [86]. In addition to the α-subunit, Gβ- and Gγ-subunits of a functional heterotrimeric G-proteins have also been identified, including Gβ1, Gβ3, and Gγ13 [93]. Other downstream components of the signaling cascade include the effector enzyme phospholipase Cβ2 (PLCβ2), the inositol 1,4,5-triphosphate (IP3) receptor IP3R3, and the transient receptor potential ion channel TRPM5 [94–97]. Null mutations of the genes for some of these signaling components abolish the animals' sensitivity to bitter substances.

Whereas sweetness and umami are attractive tastes indicative of high-calorie and nutritious foods, bitterness warns against toxic substances, causing aversion in humans and animals. In contrast to receptors for sweet and umami tastes, which have evolved to recognize a limited subset of nutrients, bitter taste receptors have the onerous task of preventing the ingestion of a large number of structurally distinct toxic compounds. Therefore, it is reasonable that bitter receptors would be encoded by a large family of genes and that the bitter sensation evolved to recognize a wide range of chemicals. A multigene family of TRC-expressed GPCRs consisting of 25 T2R members has been identified in the human genome database [98]. *In situ* hybridization has demonstrated that these receptors are expressed in a subset of TRCs. A

TRC has many of these T2Rs, and each of these receptors could be specifically activated by only a few bitter compounds with high sensitivity to more bitter and usually toxic compounds than those being detected by a single T2R.Therefore, while a single T2R may be able to recognize only a few bitter substances with high specificity and sensitivity, a TRC with multiple T2Rs is tuned to respond to more bitter and usually toxic compounds than those being detected by a single T2R without compromising sensitivity.

The human T2R gene repertoire comprises 25 functional T2R genes and 8–11 nonfunctional sequences that contain premature stop codons, referred to as pseudogenes [99–101]. T2Rs are members of the seven-transmembrane domain GPCR superfamily. In contrast with the T1Rs, which have a long extracellular N-terminal domain, the bitter taste receptors have a short extracellular N-terminus [86]. The amino acid sequences of 25 functional human T2Rs (hT2Rs) are aligned in Figure 2. Individual members of the hT2R family exhibit 25–89% amino acid identity [102], and the transmembrane segments and intracellular loops share highly conserved sequence motifs. The divergence among hT2Rs in the extracellular segments reflects the high degree of variability among T2Rs needed to recognize many structurally diverse ligands [98].

Heterologous expression and ligand screening using human embryonic kidney cells have identified bitter compounds for several receptors. Mouse T2R5 (mT2R5) and rat T2R9 respond to cycloheximide [103–105]. hT2R4 and mT2R8 both respond to denatonium and 6-*n*-propyl-2-thiouracil [103]. hT2R7 is broadly tuned for response to strychnine, quinacrine, chloroquine, and papaverine [104]. hT2R10 responds selectively to strychnine [105]. hT2R16 is a candidate receptor for β-glucopyranosides (salicin and related compounds) [104]. hT2R14 is a candidate receptor for (–)-α-thujone picrotoxinin [106]. Some hT2R38 variants respond to PTC and PROP [107, 108]. hT2R43 and hT2R47 are receptors for 6-nitrosaccharin, aristolochic acid, and denatonium [102, 104]. hT2R43 and hT2R44 respond to bitterness of saccharin [109]. hT2R46 is a broadly tuned bitter receptor for sesquiterpene lactones, strychnine, and so on [110]. And hT2R1 is tentatively a receptor for bitter peptides [111].

Because of the large family of bitter receptor genes that evolved to recognize bitter sensation from of a wide range of chemicals, identification of ligands for each bitter receptor is necessary to establish the entire bitter tasting profile for humans. However, more than half of the putative hT2Rs still remain orphan despite the fact that some of them have been characterized, i.e., correlated with their ligands. The amino acid sequence identities of hT2R43 to hT2R47 and hT2R44 are 82% and 89%, respectively. However, hT2R44 is not activated by either 6-nitrosaccharin or denatonium, whereas hT2R47 and hT2R43 are. The sequence comparison among the receptors and GTPγS binding assay using their mutants demonstrated that the first extracellular loop of the receptor is critical for the receptor activation by ligands, whereas the third extracellular loop probably is not involved in signaling by the ligands [102].

Substantial variation in human taste abilities has also been found among different individuals [112, 113]. Single nucleotide polymorphisms (SNPs) have been found in many T2R genes in many individuals. The human population exhibits unusually high levels of allelic variations in hT2R genes compared to other genes. Polymorphisms in hT2R38 influence individual differences in the ability to sense phenylthiocarbamide (PTC) and propylthiouracil, compounds that contain the N–C=S moiety [107]. Although some subjects could not identify the taste of PTC as being strongly bitter, most of them did. The inability of humans to taste PTC is due to a failure of G-protein activation rather than decreased binding affinity of the receptor for PTC, based on the computational prediction that PTC binds at a site distant from the variant amino acids. PTC-binding energy was equivalent for both the taster and nontaster forms of the protein [114]. In the case of hT2R16, a coding SNP (cSNP), K172N, was significantly associated with alcohol dependence [115]. The K172N substitution is located in extracellular loop 2 between transmembrane domains 4 and 5. Experimental evidence has shown that extracellular loop 2 is involved in the activation of hT2R43 by its agonist 6-nitrosaccharin [102], which suggests that the K172N substitution in extracellular loop 2 may alter receptor signaling or taste perception.

T2Rs have divergent regions in the extracellular loops, whereas the highly conserved sequence motifs are located in the transmembrane domains and intracellular loops [97]. When the amino acid sequences of all 25 hT2Rs are aligned, a consensus site for Asn-linked glycosylation (Asn-Xaa-Ser/Thr) is evident within the second extracellular loop, which is believed to be of functional importance [116]. Many cell surface proteins, including receptors, are Asn-linked glycosylated. For some of these proteins, glycosylation is essential for efficient plasma membrane targeting [117], whereas for other proteins, cell surface export is independent of glycosylation [118]. Experiments using receptor constructs that were point-mutated to remove various consensus sequences for Asn-linked glycosylation and using the glycosylation inhibitor tunicamycin have shown a strong functional impair-

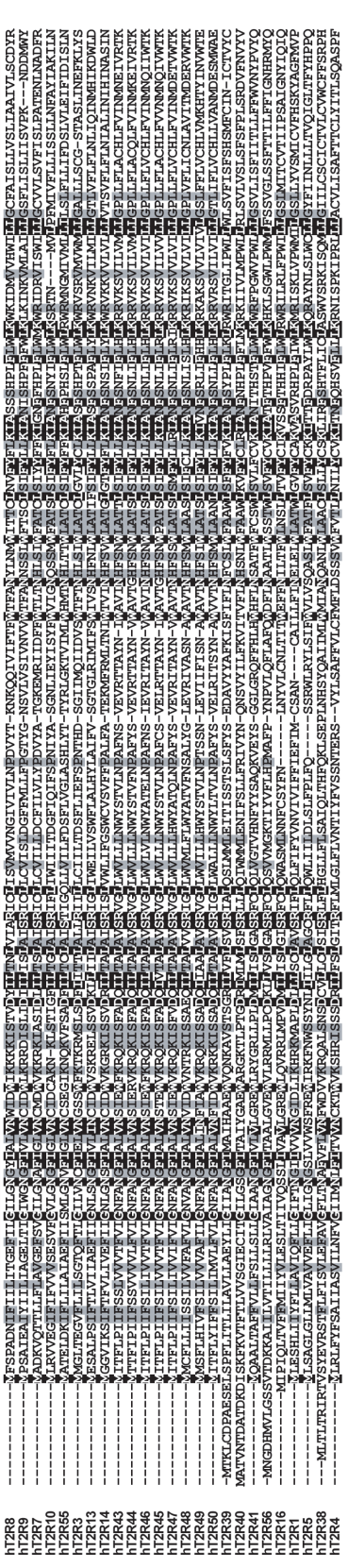

Figure 2. Sequence alignment of hT2Rs. Predicted amino acid sequences of 25 hT2Rs are aligned with CluctalW. Residues highlighted in black and gray are identical in at least 75% and 50%, respectively, of the 25 sequences. Predicted topology is indicated based on the hT2R16 sequence [115]: TM, transmembrane domain; EL, extracellular loop domain; IL, intercellular loop domain. TMs and ILs are highly conserved, whereas ELs are divergent among the 25 functional T2Rs. There are potential *N*-glycosylation motifs in EL1, and the most conserved in EL2.

ment of hypoglycosylated hT2Rs. Although hT2R16 and hT2R46 have only a single glycan structure, two glycosylation sites appear to influence the function of hT2R14. Because nonglycosylated receptors have a substantially lower cell surface localization and reduced association with the cellular chaperone calnexin, it is believed that Asn-linked glycosylation is important for receptor maturation but not for its ability to recognize bitter substances [116].

Some structural requirements of pyranoside compounds that activate hT2R16 have also been investigated [105]. Both the β-configuration of the glycosidic bond and the steric position of the hydroxyl group at C4 of pyranose are equally crucial for activation of hT2R16. The chemical groups present at C1 are also important but not essential. The aromatic phenyl ring has better agonist properties than does a small methyl group. Hydrophilic substitutions on the aromatic rings correlate inversely with agonist potency. Substitutions at C6 have only a small effect on receptor activation. The bitter unit comprises a hydrogen acceptor and donor site formed by two of the hydroxyl groups of the glucose moiety. The bitter unit (BU) is thought to work like a "hook" in attaching itself to the receptor, thereby bringing the hydrophobic portion of the molecule into the acceptor site. Phenyl-α-D-glucopyranoside and phenyl-β-D-galactopyranoside do not activate hT2R16, whereas β-glucopyranosides such as salicin do. These structurally related compounds activate separate receptors, demonstrating the complexity of deciphering the receptor–ligand relationships that comprise bitter taste.

Perspectives

Although animals encounter many structurally diverse bitter compounds, to date only 25 functional bitter receptors have been identified in the human genome. Recent molecular and functional data have shown that some receptors recognize a wide variety of bitter compounds, while other receptors are more specific. These 25 hT2Rs may, in a complementary manner, recognize a specific chemical structure for each receptor to perceive numerous bitter substances. For example, casein hydrolysate, which contains numerous bitter peptides with different structures, can activate several T2Rs [111]. Moreover, two bitter peptides, Gly-Phe and Gly-Leu, also activate all hT2R-expressing cells tested, whereas a nonbitter peptide, Gly-Gly, does not.

Ishibashi et al. [81] proposed that the side-chain skeleton of the amino acid component of Gly-Phe and Gly-Leu had to consist of at least three carbons for bitter taste to be experienced, and that the intensity of bitterness is primarily affected by the number of carbons responsible for the hydrophobicity of the side chain. However, the structural features required for a compound to be classified as bitter still have not been fully elucidated, and many receptors have yet to be deorphanized.

Bitter peptides are an important tool in the systematic analysis of the structure-function relationship between bitter peptides and bitter receptors, because of their structurally diverse nature. Knowledge of the structural features of bitter receptors and of the factors that stimulate them will aid in understanding the mechanisms responsible for bitter taste perception.

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1 Mazur, R. H., Schlatter, J. M., Goldkamp, A. M. (1969) Structure-taste relationships of some dipeptides. J. Am. Chem. Soc. 91, 2684–2691.
2 Noguchi, M., Arai, S., Yamashita, M., Kata, H., Fujimaki, M. (1975) Isolation and identification of acidic ologopeptides occuring in a flavor potentiating fraction from a fish protein hydrolysate. J. Agric. Food Chem. 23, 49–53.
3 Yamasaki, Y., Maekawa, K. (1978) A peptide with delicious taste. Agric. Biol. Chem. 42, 1761–1765.
4 Yamasaki, Y., Maekawa, K. (1980) Synthesis of a peptide with delicious taste. Agric. Biol. Chem. 44, 93.
5 Tamura, M., Nakatsuka, T., Tada, M., Kawasaki, Y., Kikuchi, Y., Oka, H. (1989) The relationship between taste and primary structure of 'delicious peptide' (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) from beef soup. Agric. Biol. Chem. 53, 319–325.
6 Nakata, T., Takahashi, M., Nakatani, M., Kuramitsu, R., Tamura, M. (1995) Role of basic and acidic fragments in delicious peptides (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) and the taste behavior of sodium and potassium salts in acidic ologopeptides. Biosci. Biotechnol. Biochem. 59, 689–693.
7 Wang, K., Mega, J. A., Bechtel, P. J. (1996) Taste properties and synergisms of beefy meaty peptide. J. Food Sci. 61, 837–839.
8 Kirimura, J., Shimizu, A., Kimizuka, A., Ninomiya, T., Katsuya, N. (1969) The contribution of peptides and amino acids to the taste of foodstuffs. J. Agric. Food Chem. 17, 689–695.
9 Maehashi, K., Matsuzaki, M., Yamamoto, Y., Udaka, S. (1999) Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. Biosci. Biotechnol. Biochem. 63, 555–559.
10 Ishibashi, N., Kouge, K., Shinoda, I., Kanehisa, H. and Okai, H. (1988) A mechanism for bitter taste sensibility in peptides. Agric. Biol. Chem. 52, 819–827.
11 Margolskee, R. F. (2002) Molecular mechanisms of bitter and sweet taste transduction. J. Biol. Chem. 277, 1–4.
12 Montmayeur, J-P., Matsunami, H. (2002) Receptors for bitter and sweet taste. Current Opinion in Neurobiology. 12, 366–371.
13 Mueller, K. L., Hoon, M. A., Erlenbach, I., Chandrashekar, J., Zuker, C. S., Ryba, N. J. P. (2005) The receptors and coding logic for bitter taste. Nature. 434, 225–229
14 Behrens, M., Meyerhof, W. (2006) Bitter taste receptors and human bitter taste perception. Cell. Mol. Life. Sci. 63, 1501–1509.

15 Sugita, M. (2006) Taste perception and coding in the periphery. Cell. Mol. Life. Sci. 63, 2000–2015.
16 Chandrashekar, J., Hoon, M. A., Ryba, N. J. P., Zuker, C. S. (2006) The receptors and cells for mammalian taste. Nature. 444, 288–294.
17 Breslin, P.A. and Huang, L. (2006) Human taste: peripheral anatomy, taste transduction and coding. In: Adv. Otorhinolaryngol. Vol. 63, pp.152–190, Hummel, T., Welge-Lussen, A. (ed.), Karger, Basel.
18 Takeuchi, T., Yoshii, H. (1969) Studies on peptides in miso and soy-sauce (VI). Group separation of lower peptides in mame-miso and their properties. J. Ferment. Technol. 47, 496–501.
19 Oka, S., Nagata, K. (1974) Isolation and characterization of neutral peptides in soy sauce. Agric. Biol. Chem. 38, 1185–1194.
20 Oka, S. Nagata, K. (1974) Isolation and characterization of acidic peptides in soy sauce. Agric. Biol. Chem. 38, 1195–1202.
21 Park, J-N., Ishida, K., Watanabe, T., Endoh, K., Watanabe, K., Murakami, M., Abe, H. (2002) Taste effects of oligopeptides in a Vietnamese fish sauce. Fisheries Sci. 68, 921–928.
22 Yamasaki, Y. (1987) The bitter taste in natto. J. Home Econ. Jpn. 38, 93–97.
23 Hosokawa, M., Sakakibara, H., Yajima, I., Hayashi, K. (1990) Non-volatile flavor components in dorsal, abdominal and red meat part of dried skipjack (Katsuobushi). Nippon Shokuhin Kogyo Gakkaishi 37, 856–861.
24 Hamilton, J. S., Hill, R. D., Van-Leeuwen, H., (1994) A bitter peptide from cheddar cheese. Aric. Biol. Chem. 38, 375–379.
25 Fernandez, M., Singh, T. K., Fox, P. F. (1998) Isolation and identification of peptides from the diafiltration permeate of the water-soluble fraction of cheddar cheese. J. Agric. Food Chem. 46, 4512–4517.
26 Mojarro-Guerra, S., Amado, R., Arrigoni, E., Solms, J. (1991) Isolation of low-molecular-weight taste peptides from Vacherin Mont d'Or cheese. J. Food Sci. 56, 943–947.
27 Broadbent, J. R., Strickland, M. S., Weimer, B. C., Johnson, M. E., Steele, J. L. (1998) Peptide accumulation and bitterness in cheddar cheese made using single-strain Lactococcus lactis starters with distinct proteinase specificities. J. Dairy. Sci. 81, 327–337.
28 Takahashi, K., Tadenuma, M., Kitamoto, K., Sata, S. (1974) L-Prolyl-L-leucine anhydride. A bitter compound formed in aged sake. Agric. Biol. Chem. 38, 927–932.
29 Hashizume, K., Okuda, M., Numata, M., Iwashita, K. (2007) Bitter-tasting sake peptides derived from the N-terminus of the rice glutelin acidic subunit. Food Sci. Technol. Res. 13, 270–274.
30 Yamashita, M., Arai, S., Fujimaki, M. (1969) Applying proteolytic enzymes on soybean. Part IV. A ninhydrin-negative bitter peptide in peptic hydrolyzate of soybean protein. Agric. Biol. Chem. 33, 321.
31 Matoba, T., Hayashi, R., Hata, T. (1970) Isolation of bitter peptides from tryptic hydrolysate of casein and their chemical structure. Agric. Biol. Chem. 34, 1235–1243.
32 Minamiura, N., Matsumura, Y., Fukumoto, J., Yamamoto, T. (1972) Bitter peptides in cow milk casein digests with bacterial proteinase. Agric. Biol. Chem. 36, 588–595.
33 Maehashi, K., Arai, S. (2002) Taste evaluation for peptides in protein hydrolysates from soybean and other plants. In: Molecular methods of plant analysis, Vol. 21, pp.47–68. Jackson, J. F., Linskens, H. F. (ed.), Springer, Berlin.
34 Murray, T. Baker, B. E. (1952) Studies on protein hydrolysis. I Preliminary observations and the taste of enzymic protein-hydrolysates. J. Sci. Food Agric. 3, 470.
35 Raadsvelt, C. W. (1953) Bitter compounds from cheese. In: Proc. 13th Int.Dairy Congr. Publ. 2, p676.
36 Mennella, J. A., Griffin, C. E., Beauchamp, G. K. (2004) Flavor programming during infancy. Pediatrics 113, 840–845.
37 Mennella, J. A., Beauchamp, G. K. (1991) Maternal diet alters the sensory qualities of human milk and the nursling's behavior. Pediatrics 88, 737–744.
38 Liem, D. G., Mennella, J. A. (2002) Sweet and sour preferences during childhood: role of early experiences. Dev. Psychobiol. 41, 388–395.
39 Mennela, J. A., Beauchamp, G. K. (2005) Understanding the origin of flavor preferences. Chem. Senses 30, i242-i243.
40 Kimball, B. A., Nolte, D. L., Perry, K. B. (2005) Hydrolyzed casein reduces browsing of trees and shrubs by white-tailed deer. Hortscience 40, 1810–1814.
41 Kimball, B. A., Nolte, D. L. (2006) Development of a deer repellent for the protection of forest resources. Western Journal of Applied Forestry 21, 108–111.
42 Kimball, B. A., Russell, J. H., DeGraan, J. P., Perry, K. R. (2008) Screening hydrolyzed casein as a deer repellent for reforestation applications. Western Journal of Applied Forestry 23, 172–176.
43 Figueroa, J. A., Kimball, B. A., Perry, K. R. (2008) Lagomorph and rodent repsonses to two protein hydrolysates. Crop Protection 27, 851–854.
44 Field, K. L., Kimball, B. A., Mennella, J. A., Beauchamp, G. K., Bachmanov, A. A. (2008) Avoidance of hydrolyzed casein by mice. Physiol. Behav. 28, 189–199.
45 Wang, W., de Mejia, G. (2005) A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. Comprehensive Reviews in Food Science and Food Safety. 4, 63–78.
46 Kim, M. R., Choi, S. Y., Lee, C. H. (1999) Molecular characterization and bitter taste formation of tryptic hydropysis of 11 S glycinin. J. Microbiol. Biotechnol. 9, 509–513.
47 Arai, S., Noguchi, M., Kurosawa, S., Kato, H., Fujimaki, M. (1970) Applying proteolytic enzymes on soybean. 6. Deodorization effect of Aspergillopeptidase A and debittering effect of *Aspergillus* acid carboxypeptidase. J. Food Sci. 35, 392–395.
48 Fiat, A-M., Jolles, P. (1989) Caseins of various origins and biologically active casein peptides and oligosaccharides: Structural and physiological aspects. Mol. Cell. Biochem. 87, 5–30.
49 Meisel, H. (1998) Overview on milk protein-derived peptdies. Int. Dairy J. 8, 363–373.
50 Shah, N. P. (2000) Effects of milk-derived bioactives: an overview. British Journal of Nutrition 84, S3-S10.
51 Yoshikawa, M., Fujita, H., Matoba, N., Takenaka, Y., Yamamoto, T., Yamauchi, R., Tsuruki, H., Takahata, Y. (2000) Bioactive peptides derived from food proteins preventing lifestyle-related disieases. Biofactors 12, 143–146.
52 Arai, S., Osawa, T., Ohigashi, H., Yoshikawa, M., Kaminogawa, S., Watanabe, M., Ogawa, T., Watanabe, S., Nishino, H., Shinohara, K., Esashi, T., Hirahara, T. (2001) A mainstay of functional food science in Japan-history, present status, and future outlook. Biosci. Biotechnol. Biochem. 65, 1–13.
53 Silva, S., Malcata, F. X. (2004) Caseins as source of bioactive peptides. International Dairy Journal 15, 1–15.
54 Meisei, H., Bockelmann, W. (1999) Bioactive peptides encrypted in milk proteins: proteolytic activation and thropho-functional properties. Antone van Leeuwenhoek 76, 207–215.
55 Li, G-H., Le, G-W., Shi, Y-H., Shrestha, S. (2004) Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. Nutr. Res. 24, 469–486.
56 Pripp, A. H., Ardo, Y. (2007) Modelling relationship between angiotensin-(I)-converting enzyme inhibition and the bitter taste peptides. Food Chem. 102, 880–888.
57 Ney, K. H. (1971) Prediction of bitterness of peptides from their amino acid composition. Z. Lebensm Unters Forsch. 147, 64–71.
58 Guigoz, Y., Solms, J. (1976) Bitter peptides, occurrence and structure. Chemical Senses and Flavor 2, 71–84.

59 Tanford, C. (1962) Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. J. Am. Chem. Soc. 84, 4240.
60 Toelstede, S., Hofmann, T. (2008) Sensomics mapping and identification of the key bitter metabolites in Gouda cheese. J. Agric. Food Chem. 56, 2795–2804.
61 Shallenberger, R. S. (1963) Hydrogen bonding and the varying sweetness of sugars. J. Food Sci., 28, 584–589.
62 Shallenberger, R. S., Acree, T. E. (1967) Molecular theory of sweet taste. Nature 216, 480–482.
63 Shallenberger, R. S., Acree, T. E. (1969) Molecular structure and sweet taste. J. Agric. Food Chem. 17, 701–703.
64 Bumberger, E. and Belitz, H-D. (1993) Bitter taste of enzymic hydrolysates of casein. I. Isolation, structural and sensorial analysis of peptides from tryptic hydrolysates of β-casein. Z. Lebensm. Unters. Forsch. 197, 14–19.
65 Fukui, H., Kanehisa, H., Ishibashi, N., Miyake, I., Okai, H. (1983) Studies of bitter peptides from casein hydrolyzate. I. Synthesis of bitter peptide BPIa corresponding to Arg-Gly-Pro-Pro-Phe-Ile-Val from casein hydrolyzate by alkaline proteinase of Bacillus subtillis. Bull. Chem. Soc. Jpn. 56, 766–769.
66 Otagiri, K., Miyake, I., Ishibashi, N., Fukui, H., Kanehisa, H., Okai, H. (1983) Studies of bitter peptides from casein hydrolyzate. II. Syntheses of bitter peptide fragments and analogs of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) from casein hydrolyzate. Bull. Chem. Soc. Jpn. 56, 1116–1119.
67 Miyake, I., Kouge, K., Kanehisa, H. and Okai, H. (1983) Studies of bitter peptides from casein hydrolysate. III. Bitter taste of synthetic analogs of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) containing D-proline or glycine in place of L-proline. Bull. Chem. Soc. Jpn. 56, 1678–1681.
68 Otagiri, K., Shigenaga, T., Kanehisa, H., Okai, H. (1984) Studies of bitter peptides from casein hydrolyzate. IV. Relationship between bitterness and hydrophobic amino acids moiety in the C-terminal of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val). Bull. Chem. Soc. Jpn. 57, 90–96.
69 Kanehisa, H., Okai, H. (1984) Studies of bitter peptides from casein hydrolyzate. V. Bitterness of the synthetic N-terminal analogs of des-Gly^{2}-BPI (Arg-Pro-Pro-Phe-Ile-Val). Bull. Chem. Soc. Jpn. 57, 301–302.
70 Kanehisa, H. (1984) Studies of bitter peptides from casein hydrolyzate. VI. Syntheses and bitter taste of BPIc (Val-Tyr-Pro-Phe-Pro-Pro-Gly-Ile-Asn-His) and its analogs and fragments. Bull. Chem. Soc. Jpn. 57, 97–102.
71 Shigenaga, K., Otagiri, K., Kanehisa, H., Okai, H. (1984) Studies of bitter peptides from casein hydrolyzate. VII. Bitterness of the retro-BPIa (Val-Ile-Phe-Pro-Pro-Gly-Arg) and its fragments. Bull. Chem. Soc. Jpn. 57, 103–107.
72 Miyake, I., Kouge, K., Kanehisa, H., Okai, H. (1984) Studies of bitter peptides from casein hydrolyzate. VIII. Bitter taste of cyclic analogs of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val). Bull. Chem. Soc. Jpn. 57, 1163–1164.
73 Miyake, I., Kouge, K., Kanehisa, H., Okai, H. (1984) Studies of bitter peptides from casein hydrolyzate. IX. Syntheses and bitter taste of bitter peptide BPIa dimer, (Arg-Gly-Pro-Pro-Phe-Ile-Val)2, and Gly-Gly BPIa derivatives. Bull. Chem. Soc. Jpn. 57, 815–818.
74 Kanehisa, H., Miyake, I., Okai, H., Aoyagi, H., Izumiya, N. (1984) Studies of bitter peptides from casein hydrolyzate. X. Synthesis and bitter taste of H-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH corresponding to C-terminal portion of β-casein. Bull. Chem. Soc. Jpn. 57, 819–822.
75 Otagiri, K., Nosho, Y., Shinoda, I., Fukui, H. and Okai, H. (1985) Studies on a model of bitter peptides including arginine, proline and phenylalanine residues. I. Bitter taste of di- and tripeptides, and bitterness increase of the model peptides by extension of the peptides chain. Agric. Biol. Chem. 49, 1019–1026.
76 Nosho, Y., Otagiri, K., Shinoda, I. and Okai, H. (1985) Studies on a model of bitter peptides including arginine, proline and phenylalanine residues. II. Bitterness behavior of a tetrapeptide (Arg-Pro-Phe-Phe) and its derivatives. Agric Biol. Chem. 49, 1829–1837.
77 Shinoda, I., Fushimi, A., Kato, H., Okai, H. and Fukui, S. (1985) Bitter taste of synthetic C-terminal tetradecapeptide of bovine β-casein, H-Pro^{196}-Val-Leu-Gly-Pro- Val-Arg-Gly-Pro-Ile-Ile-Val^{209}-OH, and its related peptides. Agric. Biol. Chem. 49, 2587–2596.
78 Shinoda, I., Nosho, Y., Kouge, K., Ishibashi, N., Okai, H., Tatsumi, K. and Kikuchi, E. (1987) Variation in bitterness potency when introducing Gly-Gly residue into bitter peptides. Agric. Biol. Chem. 51, 2103–2110.
79 Ishibashi, N., Arita, Y., Kanehisa, H., Kouge, K., Okai, H. and Fukui, S. (1987) Bitterness of leucine-containing peptides. Agric. Biol.Chem. 51, 2389–2394.
80 Ishibashi, N., Sadamori, K., Yamamoto, O., Kanehisa, H., Kouge, K., Kikuchi, E., Okai, H. and Fukui, S. (1987) Bitterness of phenylalanine- and tyrosine-containing peptides. Agric. Biol. Chem. 51, 3309–3313.
81 Ishibashi, N., Ono, I., Kato, K., Shigenaga, T., Shinoda, I., Okai, H. and Fukui, S. (1988) Role of the hydrophobic amino acid residue in the bitterness of peptides. Agric. Biol. Chem. 52, 91–94.
82 Ishibashi, N., Kubo, T., Chino, M., Fukui, H., Shinoda, I., Kikuchi, E., Okai, H. and Fukui, S. (1988) Taste of proline-containing peptides. Agric. Biol. Chem. 52, 95–98.
83 Roudot-Algaron, F. (1996) Le gout des acides amines, des peptides et des proteines. Lait 76, 313–348.
84 Matoba, T., Hata, T. (1972) Relationship between bitterness of peptides and their chemical structures. Agric Biol. Chem. 36, 1423–1431.
85 Tamura, M., Miyoshi, T., Mori, N., Kinomura, K., Kawaguchi, M., Ishibashi, N., Okai, H. (1990) Mechanism for the bitter tasting potency of petides using O-aminoacyl sugars as model comounds. Agric. Biol. Chem. 54, 1401–1409.
86 Hoon, M. A., Adler, E., Lindemeier, J., Battery, J. F., Ryba, N. J. P., Zuker, C. S. (1999) Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 96, 541–551.
87 Montmayeur, J-P., Liberles, S. D., Matsunami, H., Buck, L. B. (2001) A candidate taste receptor gene near a sweet taste locus. Nature Neurosci. 4, 492–498.
88 Zhao, G. Q., Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., Ryba, N. J. P., Zuker, C. S. (2003) The receptors for mammalian sweet and umami taste. Cell 115, 255–266.
89 Nelson, G., Hoon, M., Chandrashekar, J., Zhang, Y., Ryba, N. J. P., Zuker, C. S. (2001) Mammalian sweet taste receptors. Cell 106, 381–390.
90 McLaughlin, S. K., McKinnon, P. J., Margolskee, R. F. (1992) Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 357, 563–569. Chem. Senses 25, 525–531.
91 Ruiz-Avila, L., McLaughlin, S. K., Wildman, D., McKinnon, P. J., Robichon, A., Spickofsky, N., Margolskee, R. F. (1995) Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells. Nature 376, 80–85.
92 Kusakabe, Y., Yasuoka, A., Asano-Miyoshi, M., Iwabuchi, K., Matsumoto, I., Arai, S., Emori, Y., Abe, K. (2000) Comprehensive study on G-protein α-subunits in taste bud cells, with special reference to the occurrence of Gαi2 as a major Gα species.
93 Huang, L., Shanker, Y. G., Dubauskaite, J., Zheng, J. Z., Yan, W., Yan, W., Rosenzweig, S., Spielman, A. I., Max, M., Margolskee, R. F. (1999) Gγ13 colocalizes with gustducin in taste receptor cells and mediates IP_3 repsonses to bitter denatonium. Nat. Neurosci. 2, 1055–1062.
94 Rossler, P., Kroner, C., Freitag, J., Noe, J., Breer, H. (1998) Identification of a phospholipase Cβ subtype in rat taste cells. Eur. J. Cell Biol. 77, 253–261.
95 Clapp, T. R., Stone, L. M., Margolskee, R. F., Kinnamon, S. C. (2001) Immunocytochemical evidence for co-expression of type III IP3 receptor with signaling components of bitter taste transduction. BMC Neurosci. 2, 6.

96 Perez, C. A., Huang, L., Rong, M., Kozak, J. A., Preuss, A. K., Zhang, H., Max, M., Margolskee, R. F. (2002) A transient receptor potential channel expressed in taste receptor cells. Nat. Neurosci. 5,1169–1176.
97 Zhang, Y., Hoon, M. A., Chandrashekar, J., Mueller, K., Cook, B., Wu, D., Zuker, C. S., Ryba, N. J. P. (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112, 293–301.
98 Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekar, J., Ryba, N. J. P. (2000) A novel family of mammalian taste receptors. Cell 100, 693–702.
99 Shi, P., Zhang, J., Yang, H., Zhang, Y. (2003) Adaptive diversification of bitter taste receptor genes in mammalian evolution. Mol. Biol. Evol. 20, 805–814.
100 Fischer, A., Gilad, Y., Man, O., Paabo, S. (2004) Evolution of bitter taste receptors in humans and apes. Mol. Biol. Evol. 22, 432–436.
101 Go, Y., Satta, Y., Takenaka, O., Takahara, N. (2005) Lineage-specific loss of function of bitter taste receptor genes in humans and nonhuman primates. Genetics. 170, 313–326.
102 Pronin, A. N., Tang, H., Connor, J., Keung, W. (2004) Identification of ligands for two human bitter T2R receptors. Chem. Senses 29, 583–593.
103 Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., Zuker, C. S., Ryba, N. J. P. (2000) T2Rs function as bitter taste receptors. Cell 100,703–711.
104 Sainz, E., Cavenagh, M. M., Gutierrez, J., Battey, J. F., Northup, J. K. (2007) Functional characterization of human bitter taste receptors. Biochem. J. 403, 537–543.
105 Bufe, B., Hofmann, T., Krautwurst, D., Raguse, J-D., Meyerhof, W. (2002) The human TAS2R16 receptor mediates bitter taste in response to β-glucopyranosides. Nature Genet. 32, 379–401.
106 Behrens, M., Brockhoff, A., Kuhn, C., Bufe, B., Winning, M., Meyerhof, W. (2004) The human taste receptor hTAS2R14 responds to a variety of different bitter compounds. Biochem. Biophys. Res. Commun. 319, 479–485.
107 Bufe, B., Breslin, P. A., Kuhn, C., Reed, D. R., Tharp, C. D., Slack, J. P., Kim, U-K., Drayna, D., Meyerhof, W. (2005) The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr. Biol. 15, 322–327.
108 Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. (2003) Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299, 1221–1225.
109 Kuhn, C., Winnig, M., Hofmann, T., Frank, O., Behrens, M., Lewtschenko, T., Slack, J. P., Ward, C. D., Meyerhof, W. (2004) Bitter taste receptors for saccharin and acesulfame K. J. Neurosci. 24, 10260–10265.
110 Brockhoff, A., Behrens, M., Massarotti, A., Appendino, G., Meyerhof, W. (2007) Broad tuning of the human bitter taste receptor hTAS2R46 to various sesquiterpene lactones, clerodane and labdane diterpenoids, strychnine, and denatonium. J. Agric. Food Chem. 55, 6236–6243.
111 Maehashi, K., Matano, M., Wang, H., Vo, L. A., Yamamoto, Y., Huang, L. (2008) Bitter peptides activate hTAS2Rs, the human bitter receptors. Biochem. Biophys. Res. Commun. 365, 851–855.
112 Soronzo, N., Bufe, B., Sabeti, P. C., Wilson, J. F., Weale, M. E., Marguerie, R., Meyerhof, W., Goldstein, D. B. (2005) Positive selection on a high-sensitivity allele of the human bitter-taste receptor TAS2R16. Curr. Biol. 15, 1257–1265.
113 Ueda, T., Ugawa, S., Ishida, Y., Shibata, Y., Murakami, S., Shimada, S. (2001) Identification of coding single-nucleotide polymorphisms in human taste receptor genes involving bitter tasting. Biochem. Biophys. Res. Commun. 285, 147–151.
114 Floriano, W. B., Hall, S., Vaidehi, N., Kim, U., Drayna, D., Goddard III, W. A. (2006) Modelling the human PTC bitter-taste receptor interactions with bitter tastants. J. Mol. Model. 12, 931–941.
115 Hinrichs, A. L., Wang, J. C., Bufe, B., Kwon, J. M., Budde, J., Allen, R., Bertelsen, S., Evans, W., Dick, D., Rice, J., Foroud, T., Nurnberger, J., Tischfield, J. A., Kuperman, S., Crowe, R., Hesselbrock, V., Schckit, M., Almasy, L., Porjesz, B., Edenberg, H. J., Begleiter, H., Meyerhof, W., Bierut, L. J., Goate, A. M. (2006) Functional variant in a bitter-taste receptor (hTAS2R16) influences risk of alcohol dependence. Am. J. Hum. Genet. 78, 103–111.
116 116 Reichling, C., Meyerhof, W., Behrens, M. (2008) Functions of human bitter taste receptors depend on N-glycosylation. J. Neurochem. 10, 1–11.
117 Rands, E., Candelore, M. R., Cheung, A. H., Hill, W. S., Strader, C. D., Dixon, R. A. (1990) Mutational analysis of β-adrenergic receptor glycosylation. J. Biol. Chem. 265, 10759–10764.
118 Fukushima, Y., Oka, Y., Saitoh, T., Katagiri, H., Asano, T., Matsuhashi, N., Takata, K., van Breda, E., Yazaki, Y., Sugano, K. (1995) Structural and functional analysis of the canine histamine H2 receptor by site-directed mutagenesis: N-glycosylation is not vital for its action. Biochem. J. 310, 553–558.