Review

Journal of PeptideScience

Received: 21 July 2011 Revised: 3 October 2011 Accepted: 4 October 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/psc.1428

The good taste of peptides

Piero A. Temussi*

The taste of peptides is seldom one of the most relevant issues when one considers the many important biological functions of this class of molecules. However, peptides generally do have a taste, covering essentially the entire range of established taste modalities: sweet, bitter, umami, sour and salty. The last two modalities cannot be attributed to peptides as such because they are due to the presence of charged terminals and/or charged side chains, thus reflecting only the zwitterionic nature of these compounds and/or the nature of some side chains but not the electronic and/or conformational features of a specific peptide. The other three tastes, that is, sweet, umami and bitter, are represented by different families of peptides. This review describes the main peptides with a sweet, umami or bitter taste and their relationship with food acceptance or rejection. Particular emphasis will be given to the sweet taste modality, owing to the practical and scientific relevance of aspartame, the well-known sweetener, and to the theoretical importance of sweet proteins, the most potent peptide sweet molecules.
Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: bitter receptors; peptides; sweet taste receptor; umami receptor

Introduction

Peptides have a huge variety of biological functions, mainly as hormones or signalling molecules. To quote just a few of the most important classes of bioactive peptides, it is sufficient to mention opioids, antibiotics, immunostimulants, calcitonins, tachykinins, vasoactive intestinal peptides etc.... Do peptides also have a taste? Considering their name (from the Greek *πεπτίδια*, 'small digestibles'), it is somewhat ironical that their taste is generally not an issue.

It turns out that peptides generally do have a taste, covering essentially the entire range of established taste modalities: sweet, bitter, umami, sour and salty. The last two modalities are mainly due to the presence of charged terminals and/or charged side chains and thus do not reflect the electronic and/or conformational features of a specific peptide. The other three tastes (sweet, umami and bitter), linked to the rejection or acceptance of food, are represented by different families of peptides.

The crucial year for the description of the taste of peptides is 1969. Two papers summarized the knowledge on the taste of peptides in those times [1,2], whereas a third paper announced the discovery of aspartame [3]. Kirimura *et al*. [2] tested 60 different peptides and found that their taste intensities were weak when compared with those of the constituent simple amino acids. The taste characteristics of the studied peptides could be classified into three groups: not surprisingly, compounds in Group 1, rich in acidic residues, had a sour taste; those in Group 2, rich in hydrophobic residues, had a bitter taste; and those in Group 3, with a more balanced composition, had little or no taste. This view was essentially shared by Solms [1], who observed that not much was known about the taste of peptides but for the fact that there are no simple relations to the taste of amino acids. As pointed out above, salty and sour tastes do not reflect the electronic and/or conformational features of peptides.

At about the same time, Mazur *et al*. [3] reported that, during the synthesis of a slightly longer peptide, the C-terminal tetrapeptide of gastrin, they had serendipitously found that aspartyl-phenylalanine methyl ester was sweet, with a potency of 100–200 times that of sucrose.

Aspartame is not unique: there are many more peptides with distinct sweet, umami or bitter taste, both synthetic and naturally occurring. The recognition of naturally occurring peptides is also linked to food acceptance. This review will describe the main families of 'tasty' peptides, together with what we know about the interaction with their receptors.

Food Acceptance

Traditionally, it has been assumed that substances with a distinct taste fall into four taste categories: sweet, bitter, sour and salty. Among these four categories, sweet taste has been widely associated with food acceptance and bitter taste with food rejection. There is a fifth taste, termed umami, corresponding roughly to the taste of monosodium glutamate (MSG) and associated with the acceptance of meaty food. It was originally identified as the main taste of a typical Japanese soup prepared from sea algae and was dubbed 'delicious flavour' (umami, in Japanese) [4].

Sweet, bitter and umami tastes are the most important among the five recognized taste sensations for food acceptance or rejection. It is generally hypothesized that recognition of sweet-tasting molecules evolved to accept sugars, the body's main source of energy; umami taste evolved to recognize proteins because it is believed that the umami receptors can sense peptides or even simple amino acids derived from the hydrolysis of proteins, and the detection of bitter molecules protects humans from the ingestion of toxic compounds. It is also worth recalling that umami molecules act also as taste enhancers. Although humans, like most animals, have an innate aversion against bitter-tasting molecules, it has been observed that the rejection of bitter taste in food is not absolute. Either by cultural reasons or because of a more complex relationship among different

* *Correspondence to: Piero A. Temussi, Department of Chemistry, University of Naples "Federico II", Via Cinthia, 80126 Naples, Italy. E-mail temussi@unina.it*

MRC National Institute for Medical Research, The Ridgeway, London, UK

Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Biography

Pierandrea Temussi was born in 1939 in Nuoro (Sardinia, Italy). He graduated from the University of Naples Federico II, Naples, in 1962 and then joined the Istituto Chimico of the same university, where he worked on synthetic polymers under the supervision of P. Corradini and collaborated with G. Natta. He has worked since in several Institutions, including The Brooklyn Polytechnic (NY, USA), The Weizmann Institute of Science (Israel), University of California, San Diego (USA), University of Osaka and The National Institute for Medical Research (Medical Research Council, UK). Since 1980, he is professor of Chemistry at the University of Naples Federico II, Naples. During the period 2002–2005, he was Host Professor at the Accademia dei Lincei (Rome). His main research interests have always been centred around the use of nuclear magnetic resonance in structural biology, first on small bioactive peptides and then on globular proteins. The main scientific theme has been the study of the structure-activity relationship of sweet molecules.

tastes, humans like bitterness in certain foods, notably in beer, coffee, tea or cheese and other foods containing fermented proteins. Peptides offer some examples of chiral isomers that would be difficult to explain with a simple 'labelled line' model of taste coding [5] but open interesting possibilities when examined from the point of view of a possible cross-talk among taste cells [6].

Typical umami ligands belong to closely related chemical compounds: simple amino acids, other bifunctional acids or short peptides. On the other hand, sweet and bitter molecules belong to a very large number of diverse chemical families [7,8]. In many cases, seemingly minor constitutional modifications can change a sweet molecule into a bitter one. The most common pairs of bitter–sweet isomers are the structural isomers found in molecules containing aromatic rings [9], but in the case of peptides and amino acids, the most relevant pairs come from chiral isomerism [1]. Therefore, for almost two centuries it was believed that bitter and sweet molecules were recognized by similar mechanisms [7,9].

Most researchers interpreted the great variety of chemical classes and molecular dimensions of natural and synthetic sweet compounds in terms of the likely existence of several sweet taste receptors, and it was generally believed that one or more of these receptors had a bitter counterpart [10,11]. This view was completely abandoned when it was discovered that there is only one sweet taste receptor, the T1R2-T1R3 heterodimer [12], but several bitter taste receptors, structurally unrelated to the sweet receptor [5].

Sweet Peptides

Strictly speaking, there are no known natural peptides with a sweet taste. The only naturally occurring sweet molecules with a chemical nature of peptides are a few sweet proteins, i.e. high molecular weight polypeptides. Their biological properties do not stem from the peptide backbone, nor from the properties of one or a few side chains, but depend mainly on the shape and electronic properties of a large surface area. Thus, although proteins are indeed polypeptides, their structure–function relationship is generally treated separately from that of small molecular weight peptides.

The peptide most well known for its taste is L-aspartyl-L-phenylalanine methyl ester, i.e. aspartame, which is also the most-used-non-caloric sweetener. The accidental discovery of this sweetener [3] came as a big surprise because at the time there was no indication that peptides had a very pronounced taste, but it was soon followed by the synthesis of a very large number of dipeptide analogues, designed to improve its features as an artificial sweetener. The simple chemical constitution of aspartame and its great sweetening power stimulated an exhaustive search for analogues endowed with even higher sweetening power and, possibly, greater chemical stability.

Early on, the discoverers of aspartame explored many alternatives, trying to substitute the two amino acid residues of aspartame systematically with most naturally occurring residues. They soon found that Asp cannot be substituted by any other proteic residue, whereas Phe can be substituted by some but not any hydrophobic residue. H-Asp-Met-OMe is nearly as sweet, and H-Asp-Tyr-OMe is slightly less sweet than aspartame, but surprisingly, H-Asp-Trp-OMe is not sweet, suggesting very precise steric requirements for proper recognition [3]. Even more surprising were their findings concerning the effect, on taste, of changes at the chiral centres of either residue. All possible chiral isomers, i.e. D-L, L-D and D-D H-Asp-Phe-OMe, are bitter [3]. This finding was strongly suggestive of the existence of a 'bitter active site' structurally similar to the sweet one but with mirror image properties in part of its surface. This issue will be specifically addressed in a distinct section of this review.

In addition to the initial efforts to design analogues on the basis of mutations of Asp and/or Phe with other proteic residues [3], the discovery of aspartame stimulated extensive efforts to design small sweeteners related to aspartame only from a steric point of view but departing from the simple peptide model.

For instance, Goodman and coworkers tried to modify the nature of the amide bond [13]. Later attempts diverged even more from proteic residues, following the rationale of conventional drug design [14–16]. Suffice it to mention two original attempts to conformationally restrict the extremely flexible dipeptide, thus enhancing the natural tendencies of this peptide. The hypersweet super-aspartame analogue pCN-C_6H_4-NHCO-L-Asp-L-(αMe)Phe-OMe proved even sweeter than super-aspartame [15]. The importance of the correct topology of aspartame was further emphasized by the fact that the two diastereomeric dipeptides Ac-L-(αMe)Phe-L-Lys-OH and Ac-D-(αMe)Phe-L-Lys-OH, conformationally restricted analogues of the so-called anti-aspartame type sweeteners, proved tasteless [16].

Many of the early attempts to understand structure–taste relationships of molecules had the goal of building indirect models of the active site of the sweet receptor, which were often inspired, at least in part, by the structure of aspartame and its analogues. For instance, Temussi and coworkers [17,18] suggested a detailed model based on the superposition of rigid sweet compounds, which reflected the overall shape of the putative receptor cavity, combined also with the shape of flexible compounds, notably aspartame, whose solution structure had been previously determined [19]. Aspartame was also the starting point for the model proposed by Iwamura [20] on the basis of quantitative structure–activity relationship analyses of dipeptide analogues. Another popular topological model, mainly based on the conformation of aspartame and other dipeptides, was developed by Goodman and coworkers [21].

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Journal of PeptideScience

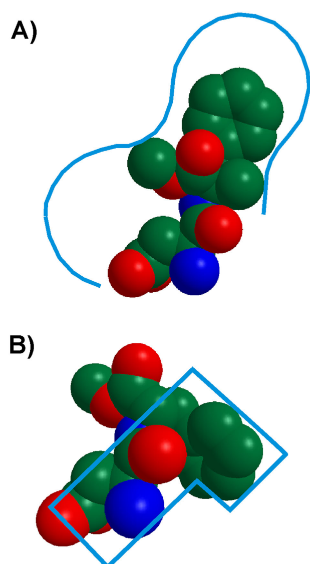

Figure 1. Indirect models of sweet active sites based on two different conformations of aspartame. (A) Temussi model with a space-filling representation of an extended conformation of [(L-α-Me)Phe[2]] aspartame. (B) Goodman 'L model' superposed to a space-filling representation of a bent conformation of aspartame.

Figure 1 shows the relationship of the models by Temussi and coworkers [18] and by Goodman and coworkers [21] with two different conformations of aspartame.

All these models have been superseded by the discovery of the sweet receptor and by the modelling of its active sites [22].

Umami Peptides

Umami taste was accepted as one of the five 'official' taste modalities later than the other four (sweet, bitter, sour and salty). Although the paradigmatic umami tastant is glutamate, many peptides have been claimed to be 'umami peptides'. Some researchers even stated that an octapeptide termed 'delicious peptide' had an umami potency higher than glutamate itself [23]. The story of umami peptides is intertwined with this octapeptide peptide, isolated by Yamasaki and Maekawa [23] from beef soup. The peptide, whose sequence is H-Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala-OH, was termed 'delicious peptide' because it produced a taste just like that of beef soup. This peptide and several segments were thoroughly examined by Tamura *et al*. [24]. Only few of the fragments examined retained some umami taste. The taste properties of the fragments that retain umami taste are reported in Table 1.

The difficulty of finding short peptides with measurable umami taste has been emphasized by the work of van den Oord and van Wassenaar [25]. They re-examined the most representative dipeptides and tripeptides reported by Tamura *et al*. [24] as well as by Ohyama *et al*. [26] and by Noguchi *et al*. [27] and found that none of the peptides had a recognizable umami taste (see Table 1). The work of van den Oord and van Wassenaar [25] is particularly careful and accurate: they tasted 19 peptides at two pH values (4.0 and 6.0) with or without 0.6% NaCl, even at concentrations much higher than those corresponding to reported thresholds. In order to test whether these peptides, like MSG itself, might have synergy with ribonucleotides, H-Glu-Glu-OH was also tasted at a concentration of 5.4 mM and pH 6.0 mixed with 0.6 mM inosine monophosphate (IMP). The taste of the mixture perceived by the panel was the same of IMP under the same conditions, i.e. a barely noticeable umami quality [25].

These observations were confirmed by Maehashi *et al*. [28], who found that the ability of several simple peptides to elicit umami taste was much less compelling than as described by Tamura *et al*. [24]. Because many hydrolysates of foodstuff containing proteins are known to possess umami taste, these authors isolated the main peptide components of the most favourable hydrolysate, i.e. the chicken protein hydrolysate obtained using bromelain. The isolated peptides, ranging from dipeptides (such as H-Asp-Glu-OH) to H-Glu-Pro-Ala-Asp-OH, had mainly a sour taste.

It seems fair to conclude that there is no convincing evidence that the small peptides suggested as umami compounds represent an independent class. Rather, it is possible that their taste is a consequence of partial hydrolysis leading to sizeable concentrations of Asp or Glu.

Bitter Peptides

Many peptides hosting residues with hydrophobic side chains have a distinct bitter taste. They have been identified in a variety of foods and, in many cases, held responsible for some of food taste qualities. In general, these peptides elicit a rather weak taste sensation, but a few have fairly high bitter potency, with thresholds only one order of magnitude lower than paradigmatic bitter compounds. Mixtures of peptides have been invoked as responsible for complex taste sensations.

Table 1. Umami peptides

Peptide	Taste	Threshold/(mM)	Reference
KGDEESLA	Umami/sour	1.41	[24]
AEA	Umami	0.8	[26]
AEA	Tasteless		[25]
GDG	Umami	1.5	[26]
GDG	Tasteless		[25]
VEV	Umami	1.5	[26]
VEV	Tasteless		[25]
EEE	Umami	300 mg%	[27]
EEE	Tasteless		[25]
KG	Salty/umami	1.22	[24]
	Slightly bitter		[25]
DE	Salty/umami	1.25	[24]
DE	Umami	1.5	[26]
DE	Tasteless		[25]
DL	Umami	2.5	[26]
DL	Tasteless		[25]
EE	Salty/umami	2.73	[24]
	Slightly bitter		[25]
EL	Umami	3.0	[26]
EL	Tasteless		[25]
EK	Umami/sour	3.12	[24]
EK	Tasteless		[25]
ED	Salty/umami	3.14	[24]
ED	Tasteless		[25]
DD	Salty/umami	4.79	[24]
DD	Tasteless		[25]

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Bitter peptides are more likely to be found in a wide variety of aged or fermented foodstuff because enzymatic hydrolysis frequently generates bitterness [29]. This finding explains also why many bitter peptides are produced during food processing as a result of the action of proteases on proteins. An exhaustive list of bitter peptides identified in early work can be found in [30].

Like in the case of umami peptides, many of the investigations looking for bitter peptides in foodstuff were performed in Japan. This circumstance explains why, beside cheese [31,32] or meaty foods, e.g. ham [33], most of the foodstuff studied belong to more or less typical Japanese food [34], such as miso [35], natto [36], katsuobushi [37], sake [38] and fish sauce [39].

In many cases, the peptides isolated from hydrolysates were not fully characterized, both for their constitution and taste qualities, and assessment of the intensity of bitter taste has been only qualitative. For instance, Fernandez *et al*. [31], who investigated the content of the water-soluble fraction of cheddar cheese, reported a series of likely peptides, characterized by N-terminal amino acid sequencing and mass spectrometry, but did not attempt to study purified peptides for their taste. Sforza *et al*. [33] isolated several peptides from extracts of Parma ham, but their identification by high-performance liquid chromatography-mass spectrometry was only tentative. Park *et al*. [39] on the other hand, after identification of 17 peptides in a Vietnamese fish sauce, did evaluate the taste of the pure, synthesized peptides but only at a single fixed concentration.

The development of bitter taste in cheese during maturation has been studied more than in other foods, possibly because of the industrial relevance of cheese. Among the proteins present in cheese, casein yields, upon hydrolysis by proteolytic enzymes, the largest number of bitter products [40]. In fact, the most important synthetic studies originated from three very bitter peptides isolated by Minamiura *et al*. [40] in cow milk casein digests: BPIa (H-Arg-Gly-Pro-Pro-Phe-Ile-Val-OH; threshold bitterness value = 0.05 mM), BPIc (H-Val-Tyr-Pro-Phe-Pro-Pro-Gly-Ile-Asn-His-OH; threshold bitterness value = 0.05 mM) and BPII. To this last peptide, the formula of a cyclic tetrapeptide was originally attributed, but it was later on found to be the simple cyclic dipeptide c(Trp-Leu), a 2,5-diketopiperazine [41]. Further characterization by Shiba *et al*. [42] showed that all four stereoisomers of this diketopiperazine, i.e. L-L, D-D, L-D and D-L, have comparable bitterness, with threshold values in the 0.03–0.06 mM range. It is noteworthy that these figures are only one order of magnitude higher than that of strychnine sulphate, one of the strongest known bitter molecules [42].

A possible explanation of this surprising lack of selectivity was provided by Goodman and Temussi [43], on the basis of an old model of the bitter active site that implied a strong resemblance between sweet and bitter receptors. This view has been apparently superseded by the discovery of a single sweet receptor and many bitter receptors that are structurally unrelated to the sweet one [5], but it has been somehow recently revisited in light of a hypothesis on pairs of bitter–sweet isomers [44].

Attempts to understand the structure–function activity of BPIa and BPIc stimulated extensive synthetic work on fragments and analogues and yielded very active bitter peptides. Otagiri and collaborators characterized both BPIa and BPIc and many fragments [45–50]. Their conclusions, based also on several model peptides, were that, in all cases, a strong bitter taste is observed when arginine is contiguous to proline such as in H-Arg-Pro-OH, H-Gly-Arg-Pro-OH and H-Arg-Pro-Gly-OH. An analogue of the parent peptide, the octapeptide (H-Arg-Arg-Pro-Pro-Pro-Phe-Phe-Phe-OH), possessed an extremely bitter taste with a threshold of 0.002 mM, comparable with that of strychnine. Table 2 compares the most important bitter peptides found by Otagiri and collaborators and some paradigmatic bitter compounds of different chemical constitution.

Starting from the structure of BPIa, BPIc and BPII, several other Japanese researchers, mainly Ishibashi and collaborators [51] and Kanehisa *et al*. [52], synthesized more than 200 synthetic peptides to study the structure–bitterness relationship of peptides. The resulting model [51,53], inspired by the earliest inductive model for sweet molecules [54], is inadequate to cope with conformational aspects of small peptides and is not consistent with findings on bitter taste receptors [5].

Receptors and Interactions

For many years, taste has been the least studied among the human senses. Thus, it is not surprising that taste receptors were characterized only recently. It was generally believed that they had to be G Protein coupled receptors (GPCRs) [55] but it was not until the year 2000 that the first receptor was identified. In this year, Chaudhari *et al*. [56] cloned and characterized a truncated version of one of the metabotrophic glutamate class C GPCRs that was proposed as a receptor for the umami taste. Soon after, Chandrashekar *et al*. [57] characterized a large family of mammalian class A GPCRs that function as bitter taste receptors (T2Rs), and Matsunami *et al*. [58] identified a family of candidate taste receptors (called TRBs).

Altogether, the best characterized taste receptors are the sweet, umami and bitter ones [5]. Among them, the sweet receptor has been more studied from the viewpoint of its interactions with many different ligands [8]. By analogy with other GPCRs, it was initially assumed that the sweet receptor had to be a homodimer of T1R3, a class C GPCR originally identified as a sweet receptor [59–65], but Li *et al*. [12] demonstrated that only heterodimer T1R2-T1R3 can properly function as a sweet receptor.

Class C GPCRs, in addition to sweet (T1R2-T1R3) and umami (T1R1-T1R3) taste receptors, include several glutamate receptors, the Ca^{2+}-sensing receptor, the α-aminobutyric acid type B

Table 2. Bitter peptides related to BPIa and BPIc*

Peptide	Taste	Threshold/(mM)
RGPPFIV (PBIa)	Bitter	0.05
VYPFPPGINH (BPIc)	Bitter	0.05
RRPPPFFF	Bitter	0.002
RPPPFFF	Bitter	0.02
RRPPFF	Bitter	0.007
RRPFF	Bitter	0.02
RPFF	Bitter	0.04
RPG	Bitter	0.8
GRP	Bitter	0.8
RP	Bitter	0.8
Caffeine	Bitter	1.0
Phenylthiourea	Bitter	0.025
Strychnine	Bitter	0.003
Brucine	Bitter	0.0008

* Otagiri *et al*. [49]

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Journal of PeptideScience

receptor and pheromone receptors [66]. Like all GPCRs, these receptors are characterized by a seven-helix transmembrane domain (7TM) but in addition have a large extracellular domain, called Venus flytrap domain (VFTD), containing the active site for typical ligands, and a cysteine-rich domain (CRD) that connects the VFTD to the 7TM domain, preventing a direct contact of the VFTD with the surface of the membrane.

Figure 2A shows a molecular representation of a dimer of a class C GPCR. The large extracellular N-terminal domain has been named VFTD after the well-known carnivorous plant.

The T1R2-T1R3 receptor can bind all sweet molecules: sugars, some D-amino acids, sweet proteins and several synthetic sweeteners [12]. The three-dimensional structure of the sweet receptor has not yet been determined. However, the high homology between the sequences of the Venus flytrap domains (VFTDs) of the two chains of the sweet receptor and that of the mGluR1 glutamate receptor, whose structure is known [67], allowed the building of homology models [22]. The X-ray structures of several forms of mGluR1 [67] hint at the existence of an equilibrium between resting and active forms of the receptor even in the absence of a ligand. It was assumed that a similar equilibrium exists also for the sweet receptor [22].

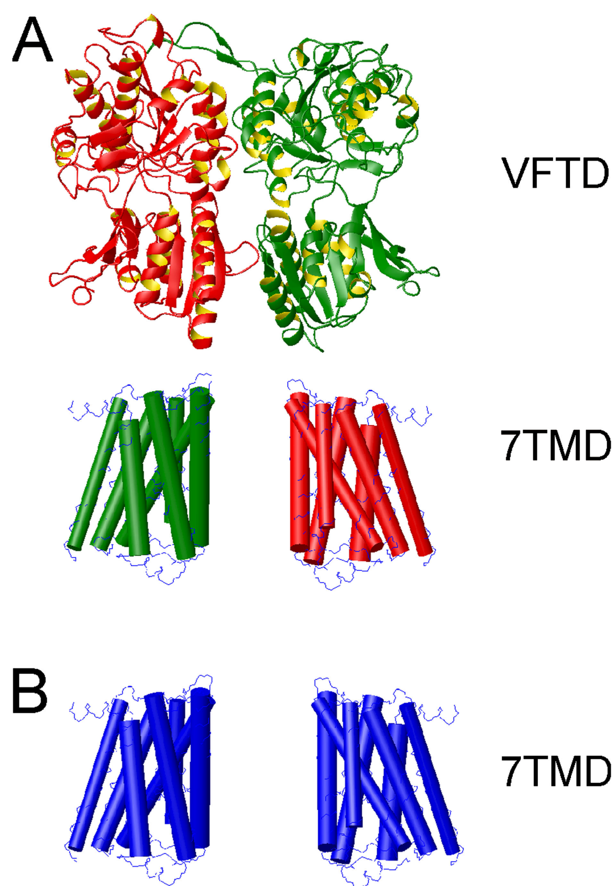

Figure 2. Molecular models of two classes of GPCRs. (A) Heterodimer of a class C GPCR, like sweet (T1R2-T1R3) or umami (T1R1-T1R3) receptors. In addition to the VFT and 7TM domains, these receptors contain a third domain (not shown), called CRD, connecting the VFTD and the 7TMD. (B) Homodimer of a generic GPCR, like the frizzled T2R bitter receptors. The 7TM domain that characterizes these receptors is similar, at low resolution, to those of class C GPCRs. Molecular models were generated by MOLMOL [83].

Cartoons illustrating these equilibria for the two receptors are shown in Figure 3. The metabotropic glutamate receptor is shown as two VFTDs of the same colour because it is a homodimer; the equilibrium between the resting open open form (Roo) and the active open closed form (Aoc) is shifted in favour of Aoc upon addition of glutamate. In the case of the sweet receptor, the T1R2 protomer is shown as a pale green VFTD, whereas the T1R3 protomer is depicted as a dark green VFTD. The active Aoc_AB form is the most likely of the active conformations described in [22]: the closed protomer is T1R2. Small molecular weight sweet molecules activate the T1R2-T1R3 receptor by shifting the equilibrium in favour of Aoc_AB.

As shown in Figure 4A, the model of [(L-α-Me)Phe^2] aspartame fits well in one of the sites of the VFTDs of the active Aoc_AB conformation of the receptor. For comparison, Figure 4B shows the fit of the same aspartame analogue in the putative site previously proposed on the basis of indirect studies [17,18].

It is not conceivable that sweet macromolecules can interact with the same active site hosting small sweeteners because the sheer size of sweet proteins is orders of magnitude larger than those of small sweeteners. For instance, it is possible to estimate the molecular volume of aspartame as 270 Å^3, whereas the molecular volume of thaumatin, the largest of known sweet proteins, is *ca* 27 000 Å^3.

A mechanism that can explain the sweetness of intensely sweet proteins, termed the 'wedge model', was proposed on

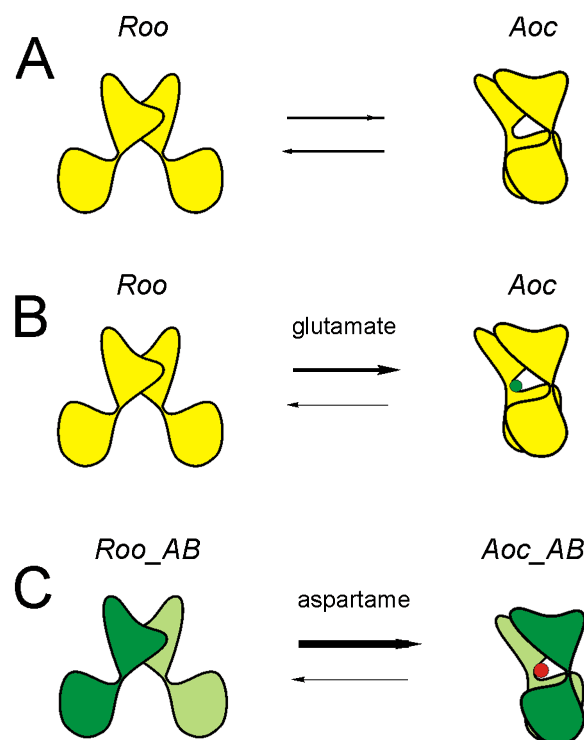

Figure 3. Schematic representation of the equilibrium between resting and active forms of the mGluR1 glutamate receptor and the corresponding equilibrium for the sweet receptor. (A) Equilibrium between Roo and Aoc of mGluR1 in the absence of a ligand. (B) Shift of the equilibrium between Roo and Aoc of mGluR1 in the presence of glutamate. (C) Shift of the equilibrium between Roo_AB and Aoc_AB of T1R2-T1R3 in the presence of aspartame. The homodimeric glutamate receptor is shown as two identical VFTs. In the case of the heterodimeric sweet receptor, the T1R2 protomer is shown as a pale grey VFT, whereas the T1R3 protomer is depicted as a dark grey VFT.

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

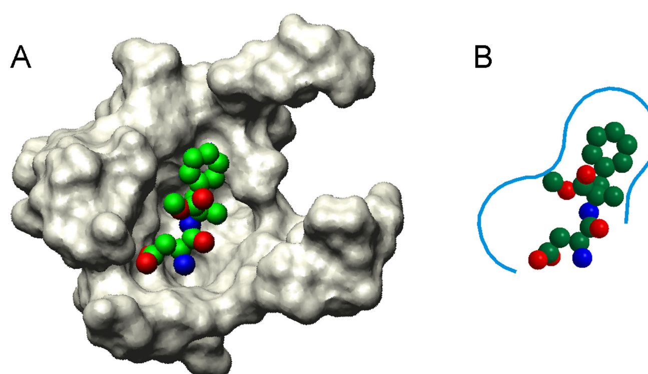

Figure 4. Comparison of the fit of [(L-α-Me)Phe[2]] aspartame in the homology-modelled sweet receptor site and in the putative site based on indirect studies. (A) Fit of [(L-α-Me)Phe[2]] aspartame in the open site of the T1R3 VFT domain of the receptor [22]. (B) Fit of the molecular model of [(L-α-Me)Phe[2]] aspartame in the putative site previously proposed on the basis of indirect studies [17,18]. The flat site is represented by the blue contour line. Molecular models were generated by MOLMOL [83].

the basis of docking calculations by using molecular structures of the three best characterized sweet proteins, i.e. brazzein, monellin and thaumatin [68].

The equilibrium between Roo_AB and Aoc_AB can be shifted in favour of Aoc_AB also by external binding of a macromolecule (Figure 5A). The large cavity on the T1R3 protomer is characterized by a negative electrostatic potential. The electrostatic potentials of the T1R3 protomer cavity and the wedge-shaped surface of MNEI (a single-chain monellin) have largely complementary charges. The complex of brazzein with the sweet receptor, as proposed by *in silico* docking, is shown in Figure 5B.

This mechanism was proposed on the basis of docking calculations by using molecular structures of the three best characterized sweet proteins, i.e. brazzein, monellin and thaumatin [68]. The model has been recently validated by a topological approach [69]. The only drawback of the wedge model, as derived from low-resolution *in silico* docking, was the fuzzy nature of low-energy complexes, which meant an intrinsic difficulty of building accurate complexes of sweet proteins with the receptor. In the quoted topological approach [69], it was shown that it is possible to obtain accurate one-to-one correspondence of receptor to sweet protein residues by yoking key mutations on the sweet proteins by means of a tethered docking procedure.

Three different receptors have been indicated as 'umami receptors': a truncated form of mGluR4 [56], the T1R1–T1R3 heterodimer [12] and a truncated form of mGluR1 [70]. Yasuo *et al*. [71] have emphasized the role of mGluRs, implying that the transduction pathway involving T1R1-T1R3 might be different from that involving mGluRs. However, the T1R1-T1R3 heterodimer is generally regarded as the prototypic umami receptor and, more in general, a receptor for all L-amino acids [5,12]. As suggested by the T1R1-T1R3 homology model [72], the active sites located in the extracellular, globular parts of T1R1 and T1R3 (called VFTD), which correspond to the Glu sites in mGluR1, should be very similar to the corresponding sites of the sweet receptor. The only existing modelling study on the umami receptor [72] does not say much on this aspect because the study is centred on the synergy between MSG and IMP. It is also not unlikely that the T1R1-T1R3 receptor might have many active sites like the T1R2-T1R3 sweet receptor [22], but the paucity of known umami ligands prevents any further speculation.

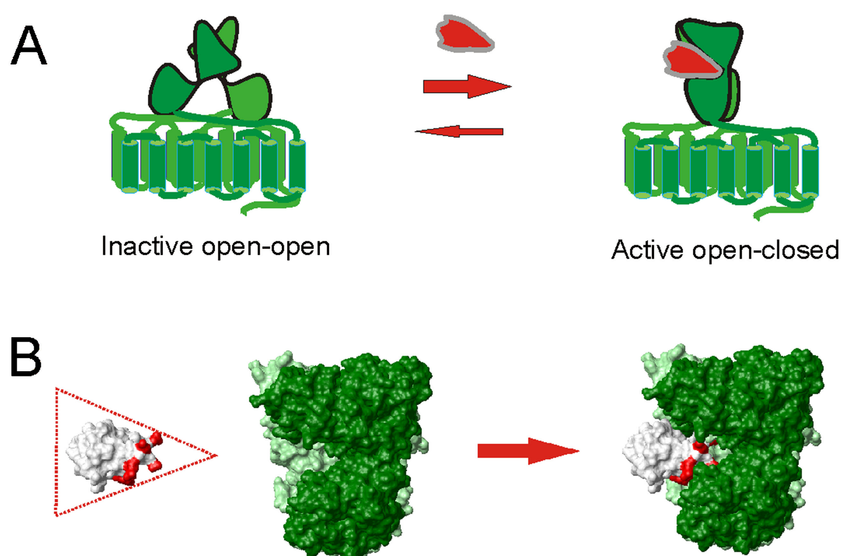

Figure 5. The wedge model for sweet proteins. (A) Schematic representation of the shift of the equilibrium between the inactive open open and the active open closed forms of T1R2-T1R3 induced by external binding of a sweet protein. (B) Binding of brazzein, represented as a wedge, and the active Aoc_AB form of the receptor. The heterodimeric sweet receptor, the T1R2 protomer, is shown as a pale green VFT, whereas the T1R3 protomer is depicted as a dark green VFT.

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Journal of PeptideScience

Compounds that elicit bitter taste are recognized by a family of class A GPCRs, collectively known as T2Rs [5]. These receptors are classified as 'frizzled' and contain a single 7TM transmembrane domain (Figure 2B). Each T2R can detect several bitter molecules, consistent with the need for most mammals to recognize a huge number of potentially dangerous bitter compounds using a relatively small number of receptors. The T2Rs, although expressed in dedicated taste receptor cells, share common signal transduction components with cells detecting sweet, umami and bitter taste qualities. These GPCRs lack the extensive N-terminal domain present in T1Rs, but it is likely that they also function as dimers.

T2Rs are 'simple' 7TM GPCRS, but a closer inspection of their sequences reveals that it is not possible to classify them in any of the most well-known groups, e.g. as class A GPCRs. Some have classified them as a separate family [73], whereas others have assigned them to the subfamily of the frizzled receptors [74]. In principle, it might be easier to classify them on the basis of detailed structure–activity studies, but studies of this kind have been scanty. Luckily, a few revealing studies have recently been published on the ligand-binding mechanisms of T2Rs [75–77].

The take-home message has been nicely summarized by Singh *et al*. [78] on the basis of accurate mutation studies, complemented by molecular modelling, on T2R1. This specific T2R is particularly relevant in the framework of the present review because it binds bitter dipeptides and tripeptides [79].

If one tries to extrapolate the results from the study on T2R1, T2Rs appear to be highly divergent from class A GPCRs, not only in their amino acid sequence but also in the mechanism of activation by natural bitter ligands. Much needed further structure–function analyses on this and other T2Rs will hopefully give detailed insights into other key residues that contribute to ligand binding.

Bitter–Sweet Pairs: Taste and Chirality

As mentioned in the Food Acceptance section, pairs of chemically related sweet and bitter compounds play a key role in the understanding of the relationship among taste qualities related to food acceptance. The most common pairs of bitter–sweet compounds come from structural isomers, congeners or even conformational analogues [7,9,44]. Some of these odd pairs and many more quoted by Moncrieff [7] and by Verkade [9] might be serendipitous, meaning that the bitter partners of bitter–sweet pairs might be recognized by one of the T2Rs, even if these receptors lack the sterical features that characterize the VFTDs of T1R2-T1R3. However, it is impossible to attribute to a purely fortuitous coincidence the taste relationship between chiral isomers. Chirality is fundamental in biology because it plays a central role in controlling molecular recognition and interactions. For instance, a clear illustration of the key role played by chirality in biologically active peptides was furnished by a study on opioid peptides [80]. It is known that the chirality of the α carbon of the tyramine moiety in morphine alkaloids is opposite with respect to that of the corresponding carbon atom in L-Tyr, the amino terminal residue in nearly all opioid peptides, a fact even more surprising because the whole molecule of morphine is essentially derived from two molecules of L-tyrosine. We hypothesized that the presence of Gly2 or D-Ala2 in the two most common message domains of opioid peptides mimics this change by allowing the attainment of unusual conformations. Indeed, a thorough conformational search of the tripeptide H-Tyr-D-Ala-Phe-NH-CH_3 and of its isomer H-Tyr-L-Ala-Phe-NH-CH_3 showed that energy-accessible conformers are consistent with the topological requirements imposed by the chirality of the α carbon of tyramine and with the very rigid constraints imposed on the tyramine moiety by cyclization [80].

The changes involving inversion of chiral centres in bitter–sweet pairs hint at very strict stereochemical requirements, implying the existence of two active sites very similar in shape but with a subtle control of chirality. Peptides and simple amino acids offer good examples of bitter–sweet pairs governed by chirality. Glycine and L-alanine are sweet, whereas most polar L-amino acids, with the exception of glutamic acid, are tasteless, and apolar amino acids are bitter [1]. An inversion of the configuration of the α carbon of apolar amino acids changes their taste from bitter to sweet: L-Trp, L-Phe and L-Tyr taste bitter (in order of decreasing bitterness), whereas D-Trp, D-Phe and D-Tyr taste sweet [1]. Even more surprising is the relationship between aspartame and its chiral isomers: H-L-Asp-L-Phe-OMe is very sweet, whereas H-D-Asp-D-Phe-OMe is bitter [3,81], as well as the two diastereomers H-D-Asp-L-Phe-OMe and H-L-Asp-D-Phe-OMe.

The molecular models of these four aspartame isomers, oriented as [(L-α-Me)Phe^2] aspartame in the sweet taste receptor [22], are shown in Figure 6. These examples indicate that it is reasonable to anticipate that the bitter receptor is structurally related to the sweet receptor and hence different from those of the T2R family.

This hypothetical receptor should be structurally similar to the T1R2-T1R3 receptor and have the ability to accept molecules with opposite chirality with respect to well-known (chiral) sweet molecules. These features are typical of T1R receptors; so we do not have to look for new members of the T1R family because such a receptor has already been described: it is T1R1-T1R3, the main umami receptor, which besides L-Glu can bind several L-amino acids [12].

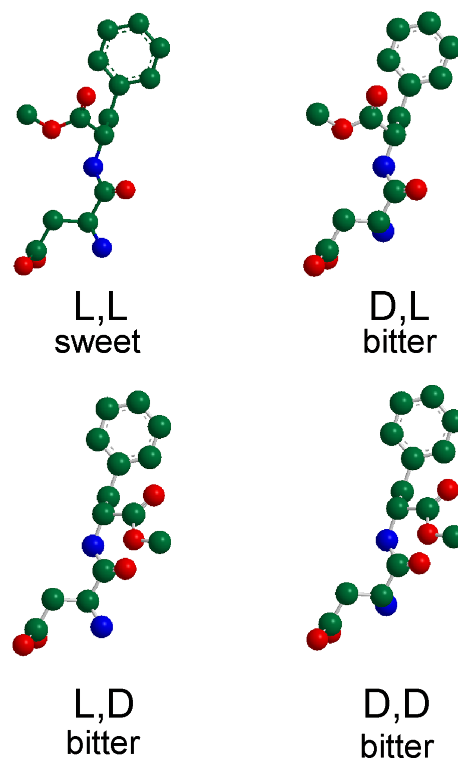

Figure 6. Molecular models of four aspartame chiral isomers, oriented as [(L-α-Me)Phe^2] aspartame in the sweet receptor [22].

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

The models of the sweet (T1R2-T1R3) and umami (T1R1-T1R3) receptors are compared in Figure 7. The structural similarity of T1R1-T1R3 with the T1R2-T1R3 sweet receptor is assured by their sequence similarity: the identity between the sequences of the different protomers of the two receptors (T1R1 and T1R2) is 37%. The ability of T1R1-T1R3 to recognize L-amino acids is well documented, whereas very sweet D-amino acids, like D-Trp, are not recognized [12].

How can a bitter peptide (related to sweet aspartame) induce bitter stimulation? The simplest explanation is that the umami receptor is hosted by a cell specialized in bitter taste: the outcome is a bitter taste coming from the stimulation of the T1R1-T1R3 receptor by the bitter component of the bitter–sweet pairs. This possibility goes against the 'dogma' of specialized taste cells [5] adopted by most molecular biologists but not by all physiologists [6]. However, Tomchik *et al*. [82] have observed that, although the majority of receptor cells are specialized to respond to sweet, bitter or umami tastes, occasionally some receptor cells can respond to two or even three taste qualities. Signalling in taste bud cells is mediated by two classes of cells, i.e. 'receptor' cells that detect and transduce sweet, bitter and umami compounds and 'presynaptic' cells. If receptor cells communicate with presynaptic cells, there is in principle a potential convergence of taste information in the taste bud, resulting in taste cells that respond to multiple taste stimuli. According to Tomchik *et al*. [82], although approximately 80% of receptor cells in taste buds do respond to only one taste stimulus, 80% of presynaptic cells (accepting signals from taste cells) respond to two or more different taste qualities, thus resolving the paradox of broad taste cell tuning despite the fact that taste cells are labelled, i.e. contain only one type of receptor each. Thus, there are three possible explanations of responses to multiple taste qualities in individual taste cells: (i) some receptor cells may host multiple taste receptors, (ii) there may be cross-talk between receptor cells or (iii) there might be a margin of uncertainty in cell classification. Possibility (ii) seems to be the most plausible explanation of the problem described here: the signals coming from two umami and bitter taste cells may converge on the same presynaptic cell. If such an umami cell is stimulated by the 'bitter partner' of one of the bitter–sweet pairs previously described, e.g. L-Trp, the signal will be interpreted as bitter. Conversely, if the tastant is L-Glu, it will stimulate a much larger number of umami taste cells, and thus the prevailing umami taste will mask any bitter aftertaste coming from the less populated umami cells connected to presynaptic cells together with a bitter cell.

Conclusion

Peptides are not very often quoted in relation with their taste, but there is little doubt that they contribute considerably to the complex taste of much of the food we eat every day, particularly in cheese and meaty food where singular mixtures of bitter and umami peptides are largely responsible for the peculiar taste of each product.

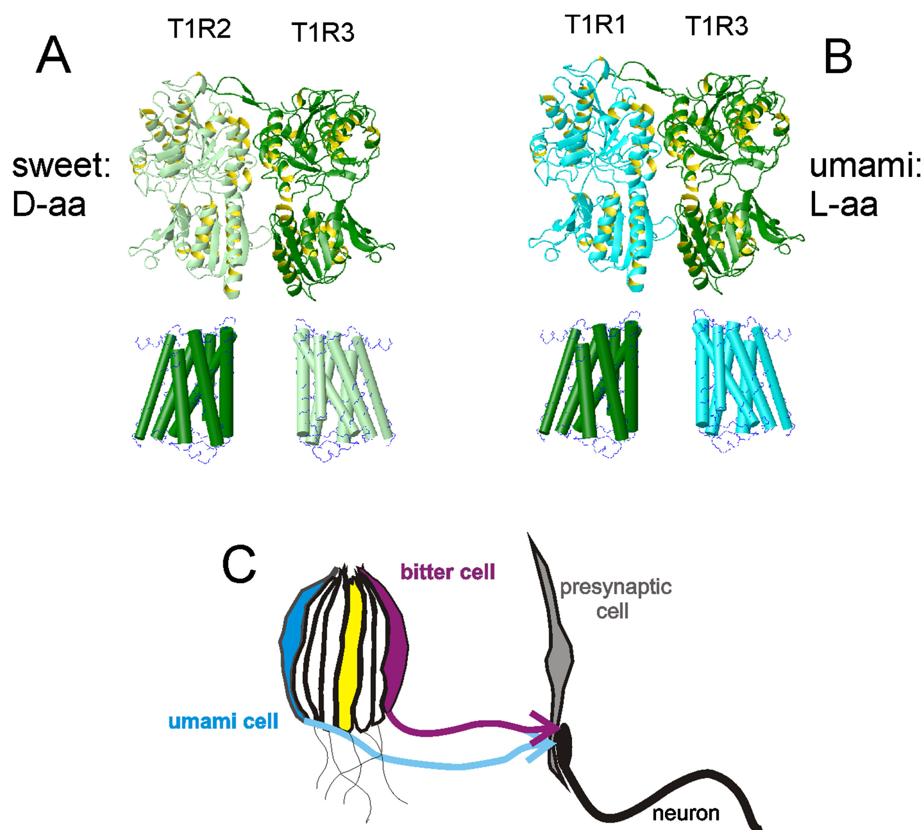

Figure 7. Bitter taste of sweet chiral isomers. (A) Heterodimeric T1R2-T1R3 sweet receptor. The T1R2 protomer is coloured in pale green; the T1R3 protomer, common to the umami receptor, is coloured in dark green. (B) Heterodimeric T1R1-T1R3 umami receptor. The T1R2 protomer is coloured in cyan; the T1R3 protomer, common to the sweet receptor, is coloured in dark green. (C) Cartoon illustrating the relationship among taste cells, which explains a possible cross-talk between umami and bitter receptor cells. Signals from two (type II) receptor cells labelled for umami (cyan) and bitter (purple) converge onto presynaptic (type III) cells (light grey). Molecular models were generated by MOLMOL [83].

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

The emphasis of this review is mainly on the sweet taste modality, both for the practical relevance of aspartame and the theoretical importance of sweet proteins. According to the proposed 'wedge model', sweet proteins, the sweetest known polypeptides, bind to an external site of the active form of the receptor. The validation of the wedge model in turn gives confidence in the reliability of the homology model of the T1R2-T1R3 receptor itself and allows its use to explain some aspects of aspartame interactions. Aspartame is at present the most widely used artificial sweetener, a critically important issue for people affected by illnesses typical of affluent society, like diabetes, caries or cardiac syndromes. It was shown that aspartame can bind to one of the VFT active sites of the sweet receptor in a manner reminiscent of the putative site derived from indirect approaches before the receptor was actually discovered. The puzzling relationship among the tastes of the four chiral isomers of aspartame was also tentatively explained on the basis of a key role of the umami receptor.

It is hoped that all above examples establish the role of peptides in the field of human taste on a firm basis. Although it is difficult to consider peptides as a class apart in the field of taste, it is clear that their chemical nature, particularly their incredible conformational versatility, plays a relevant role in determining many structure–activity relationships, including those connected to the main tastes related to food acceptance.

Acknowledgement

I wish to thank Annalisa Pastore (NIMR, MRC, London) for many useful suggestions and constant encouragement.

References

1 Solms J. Taste of amino acids, peptides, and proteins. *J. Agric. Food Chem.* 1969; **17**: 686–688.
2 Kirimura J, Shimizu A, Kimizuka A, Ninomiya T, Katsuya N. Contribution of peptides and amino acids to the taste of foods. *J. Agric. Food Chem.* 1969; **17**: 689–695.
3 Mazur RH, Schlatter JM, Goldkamp AH. Structure–taste relationships of some dipeptides. *J. Am. Chem. Soc.* 1969; **91**, 2684–2691.
4 Ikeda K. New seasonings. *Chem. Senses* 2002; **27**: 847–849.
5 Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. *Nature* 2006; **444**: 288–294.
6 Simon SA, de Araujo IE, Gutierrez R, Nicolelis MA. The neural mechanisms of gustation: a distributed processing code. *Nat. Rev. Neurosci.* 2006; **7**: 890–901.
7 Moncrieff RW. *The Chemical Senses*. Hill: London 1967.
8 Temussi PA. The sweet taste receptor: a single receptor with multiple sites and modes of interaction. *AFNR* 2007; **53**: 199–239.
9 Verkade PE. On organic compounds with a sweet and/or a bitter taste. *Il Farmaco Ed. Sc.* 1967; **23**: 248–291.
10 Beidler LM, Gross GW. The nature of taste receptor sites. In *Contributions to Sensory Physiology*, Vol. **5**, Neff WD (ed.). Academic Press: New York, N.Y., 1971; 97–127.
11 Birch GG, Mylvaganam AR. Evidence for the proximity of sweet and bitter receptor sites. *Nature* 1976; **260**: 632–634.
12 Li X, Staszewski L, Xu, H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. *Proc. Natl. Acad. Sci. USA* 2002; **99**: 4692–4696.
13 MacDonald SA, Willson CG, Chorev M, Vernacchia FS, Goodman M. Peptide sweeteners. 3. Effect of modifying the peptide bond on the sweet taste of L-aspartyl-L-phenylalanine methyl ester and its analogues. *J. Med. Chem.* 1980; **2**: 413–420.
14 Polinelli S, Broxterman QB, Schoemaker HE, Boesten WHJ, Crisma M, Valle G, Toniolo C, Kamphuis J. New aspartame-like sweeteners containing L-(αMe) Phe. *Bioorg. Med. Chem. Lett.* 1992; **2**: 453–456.
15 Toniolo C, Formaggio F, Crisma M, Valle G, Boesten WHJ, Schoemaker HE, Kamphuis J, Temussi PA, Becker EL, Precigoux G. Bioactive and model peptides characterized by the helicogenic (αMe)Phe residue. *Tetrahedron* 1992; **49**: 3641–3653.
16 Formaggio F, Crisma M, Valle G, Toniolo C, Boesten WHJ, Schoemaker HE, Kamphuis J, Temussi PA. Conformationally restricted analogues of anti-aspartame-type sweeteners. *J. Chem. Soc., Perkin Trans.* 1992; **2**: 1945–1950.
17 Temussi PA, Lelj F, Tancredi T. Three-dimensional mapping of the sweet taste receptor site. *J. Med. Chem.* 1978; **21**: 1154–1158.
18 Temussi PA, Lelj F, Tancredi T. Structure–activity relationships of sweet molecules. *In Sweeteners, Discovery, Molecular Design and Chemoreception*, Vol. **450**, Walters DE, Orthofer FT, DuBois GE (eds.). ACS Symposium Series, ACS: Washington DC, 1991; 143–161.
19 Lelj F, Tancredi T, Temussi PA, Toniolo C. Interaction of α-L-aspartyl-L-phenylalanine methyl ester with the receptor site of the sweet taste bud. *J. Am. Chem. Soc.* 1976; **98**: 6669–6675.
20 Iwamura H. Structure–sweetness relationship of L-aspartyl dipeptide analogues. A receptor site topology. *J. Med. Chem.* 1981; **24**: 572–578.
21 Yamazaki T, Benedetti E, Kent D, Goodman M. A three-dimensional model for taste utilizing stereoisomeric peptides and peptidomimetics. *Angew. Chem. Int. Ed Engl.* 1994; **33**: 1437–1451.
22 Morini G, Bassoli A, Temussi PA. From small sweeteners to sweet proteins, anatomy of the binding sites of the human T1R2-T1R3 receptor. *J. Med. Chem.* 2005; **48**: 5520–5529.
23 Yamasaki Y, Maekawa K. A peptide with delicious taste. *Agric. Biol. Chem.* 1978; **42**: 1761–1765.
24 Tamura M, Nakatsuka T, Tada M, Kawasaki Y, Kikuchi E, Okai H. The relationship between taste and primary structure of "delicious peptide" (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) from beef soup. *Agric. Biol. Chem.* 1989, **53**: 319–325.
25 Van Den Oord AHA, Van Wassenaar PD. Umami peptides: assessment of their alleged taste properties. *Eur. Food Res. Technol.* 1997; **205**: 125–130.
26 Ohyama S, Ishibashi N, Tamura M, Nishizaki H, Okai H. Synthesis of bitter peptides composed of aspartic acid and glutamic acid. *Agr. Biol. Chem.* 1988; **52**: 871–872.
27 Noguchi M, Arai S, Yamashita M, Kato H, Fujimaki MI. Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from a fish protein hydrolysate. *J. Agr. Food Chem.* 1975; **23**: 49–54.
28 Maehashi K, Matsuzaki M, Yamamoto Y, Udaka S. Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. *Biosci. Biotechnol. Biochem.* 1999; **63**: 555–559.
29 Lovsin-Kukman I, Zelenik-Blatnik M, Abram V. Bitterness intensity of soybean protein hydrolysates – chemical and organoleptic characterization. *Z. Lebensm. Unters. Forsch.* 1996; **203**: 272–276.
30 Guigoz Y, Solms J. Bitter peptides, occurrence and structure. *Chem. Senses and Flavour* 1976; **2**: 71–84.
31 Fernandez M, Singh TK, Fox PF. Isolation and identification of peptides from the diafiltration permeate of the water-soluble fraction of cheddar cheese. *J. Agric. Food Chem.* 1988; **46**: 4512–4517.
32 Broadbent JR, Strickland MS, Weimer BC, Johnson ME, Steele JL. Peptide accumulation and bitterness in cheddar cheese made using single-strain *Lactococcus lactis* starters with distinct proteinase specificities. *J. Dairy Sci.* 1998; **81**: 327–337.
33 Sforza S, Pigazzani A, Motti M, Porta C, Virgili R, Galaverna G, Dossena A, Marchelli R. Oligopeptides and free amino acids in Parma hams of known cathepsin B activity. *Food Chem.* 2001; **75**: 267–273.
34 Maehashi K, Huang L. Bitter peptides and bitter taste receptors. *Cell. Mol. Life Sci.* 2009; **66**: 1661–1671.
35 Takeuchi T, Yoshii H. Studies on peptides in miso and soy-sauce (VI). Group separation of lower peptides in mame-miso and their properties. *J. Ferment. Technol.* 1969; **47**: 496–501.
36 Yamasaki Y. The bitter taste in natto. *J. Home Econ. Jpn.* 1987; **38**: 93–97.
37 Hosokawa M, Sakakibara H, Yajima I, Hayashi K. Non-volatile flavor components in dorsal, abdominal and red meat part of dried skipjack (Katsuobushi). *Nippon Shokuhin Kogyo Gakkaishi* 1990; **37**: 856–861.
38 Maeda Y, Okuda M, Hashizume K, Joyo M, Mikami S, Goto-Yamamoto N. Analyses of peptides in sake mash: forming a profile of bitter-tasting peptides. *J. Biosci. Bioeng.* 2011; **112**: 238–246.

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

39 Park J-N, Ishida K, Watanabe T, Endoh K, Watanabe K, Murakami M, Abe H. Taste effects of oligopeptides in a Vietnamese fish sauce. *Fisheries Sci.* 2002; **68**: 921–928.
40 Minamiura N, Matsumura Y, Fukumoto J, Yamamoto T. Bitter peptides in cow milk casein digests with bacterial proteinase. *Agric. Biol. Chem.* 1972; **36**: 588–595.
41 Shiba T, Nunami K. Structure of a bitter peptide in casein hydrolyzate by bacterial proteinase. *Tetrahedron Lett.* 1974; **15**: 509–512.
42 Shiba T, Uratani H, Kubota I, Sumi Y. Some aspects of the relationship between the structure of a bitter diketopiperazine and its receptor. *Biopolymers* 1981; **20**: 1985–1987.
43 Goodman M, Temussi PA. Structure–activity relationship of a bitter diketopiperazine revisited. *Biopolymers* 1985; **23**: 2349–2360.
44 Temussi PA. Sweet, bitter and umami receptors: a complex relationship. *TiBS* 2009; **34**: 296–302.
45 Fukui H, Kanehisa H, Ishibashi N, Miyake I, Okai H. 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 subtilis*. *Bull. Chem. Soc. Jpn.* 1983; **56**: 766–769.
46 Otagiri K, Miyake I, Ishibashi N, Fukui H, Kanehisa H, Okai H. 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.* 1983; **56**: 1116–1119.
47 Otagiri K, Shigenaga T, Kanehisa H, Okai H. 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.* 1984; **57**: 90–96.
48 Shigenaga K, Otagiri K, Kanehisa H, Okai H. 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.* 1984; **57**: 103–107.
49 Otagiri K, Nosho Y, Shinoda I, Fukui H, Okai H. 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 peptide chain. *Agric. Biol. Chem.* 1985; **49**: 1019–1026.
50 Nosho Y, Otagiri K, Shinoda I, Okai H. 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.* 1985; **49**: 1829–1837.
51 Ishibashi N, Kouge K, Shinoda I, Kanehisa H, Okai H. A mechanism for bitter taste sensibility in peptides. *Agric. Biol. Chem.* 1988; **52**: 819–827.
52 Kanehisa H, Miyake I, Okai H, Aoyagi H, Izumiya N. 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.* 1984; **57**: 819–822.
53 Tamura M, Miyoshi T, Mori N, Kinomura K, Kawaguchi M, Ishibashi N, Okai H. Mechanism for the bitter tasting potency of peptides using O-aminoacyl sugars as model compounds. *Agric. Biol. Chem.* 1990; **54**: 1401–1409.
54 Shallenberger RS, Acree TE. Molecular structure and sweet taste. *J. Agric. Food Chem.* 1969; **17**: 701–703.
55 Gilbertson T, Damak S, Margolskee R. The molecular physiology of taste transduction. *Curr. Opin. Neurobiol.* 2000; **10**: 519–527.
56 Chaudhari N, Landin AM, Roper SD. A metabotropic glutamate receptor variant functions as a taste receptor. *Nat. Neurosci.* 2000; **3**: 113–119.
57 Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ. T2Rs function as bitter taste receptors. *Cell* 2000; **100**: 703–711.
58 Matsunami H, Montmayeur J-P, Buck L. A family of candidate taste receptors in human and mouse. *Nature* 2000; **404**: 601–604.
59 Bachmanov AA, Li X, Reed DR, Ohmen JD, Li S, Chen Z, Tordoff MG, de Jong PJ, Wu C, West DB, Chatterjee A, Ross DA, Beauchamp GK. Positional cloning of the mouse saccharin preference (Sac) locus. *Chem. Senses* 2001; **26**: 925–933.
60 Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem. Biophys. Res. Commun.* 2001; **283**: 236–242.
61 Li X, Inoue M, Reed DR, Hunque T, Puchalski RB, Tordoff MG, Ninomiiya Y, Beauchamp GK, Bachmanov AA. High-resolution genetic mapping of the saccharin preference locus (Sac) and the putative sweet taste receptor (T1R1) gene (Gpr70) to mouse distal chromosome 4. *Mamm. Genome* 2001; **12**: 13–16.
62 Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H, Damak S, Margolskee RF. Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. *Nat. Genet.* 2001; **28**: 58–63.
63 Montmayeur JP, Liberles SD, Matsunami H, Buck LBA. Candidate taste receptor gene near a sweet taste locus. *Nat. Neurosci.* 2001; **4**: 492–498.
64 Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. *Cell* 2001; **106**: 381–390.
65 Sainz E, Korley JN, Battey JF, Sullivan SL. Identification of a novel member of the T1R family of putative taste receptors. *J. Neurochem.* 2001; **77**: 896–903.
66 Pin JP, Galvez T, Prezeau L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol. Therap.* 2003; **98**: 325–354.
67 Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, Morikawa K. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* 2000; **407**: 971–977.
68 Temussi PA. Why are sweet proteins sweet? Interaction of brazzein, monellin and thaumatin with the T1R2-T1R3 receptor. *FEBS Lett.* 2002; **526**: 1–3.
69 Temussi PA. Determinants of sweetness in sweet proteins: how to build improved topological models from the low resolution complexes of the T1R2_T1R3 receptor with monellin and brazzein. *J. Mol. Recogn.* (in press).
70 San Gabriel A, Uneyama H, Yoshie S, Torii K. Cloning and characterization of a novel mGluR1 variant from vallate papillae that functions as a receptor for L-glutamate stimuli. *Chem. Senses* 2005; **30**: i25–i26.
71 Yasuo T, Kusuhara Y, Yasumatsu K, Ninomiya Y. Multiple receptor systems for glutamate detection in the taste organ. *Biol. Pharm. Bull.* 2008; **31**: 1833–1837.
72 Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C, Li X. Molecular mechanism for the umami taste synergism. *Proc. Natl. Acad. Sci. USA* 2008; **105**: 20930–20934.
73 Horn F, Bettler E, Oliveira L, Campagne F, Cohen FE, Vriend G. NRSAS: Nuclear Receptor Structure Analysis Servers. *Nucleic Acids Res.* 2003; **31**: 294–297.
74 Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharmacol.* 2003; **63**: 1256–1272.
75 Sakurai T, Misaka T, Ishiguro M, Masuda K, Sugawara T, Ito K, Kobayashi T, Matsuo S, Ishimaru Y, Asakura T, Abe K. Characterization of the β-D-glucopyranoside binding site of the human bitter taste receptor hTAS2R16. *J. Biol. Chem.* 2010; **285**: 28373–28378.
76 Biarnes X, Marchiori A, Giorgetti A, Lanzara C, Gasparini P, Carloni, P, Born S, Brockhoff A, Behrens M, Meyerhof W. Insights into the binding of Phenyltiocarbamide (PTC) agonist to its target human TAS2R38 bitter receptor. *PLoS One* 2010; **5**: e12394.
77 Brockhoff A, Behrens M, Niv MY, Meyerhof W. Structural requirements of bitter taste receptor activation. *P.N.A.S. USA* 2010; **107**: 11110–11115.
78 Singh N, Pydi P, Upadhyaya J, Chelikani P. Structural basis of activation of bitter taste receptor T2R1 and comparison with class A GPCRs. *J. Biol. Chem.* 2011; **286**: 36032–36041.
79 Upadhyaya J, Pydi SP, Singh N, Aluko RE, Chelikani P. Bitter taste receptor T2R1 is activated by dipeptides and tripeptides. *Biochem. Biophys. Res. Commun.* 2010; **398**: 331–335.
80 Fraternali F, Anselmi C, Temussi PA. Neurologically active plant compounds and peptide hormones: a chirality connection. *FEBS Lett.* 1999; **448**: 217–220.
81 Castiglione-Morelli MA, Lelj F, Naider F, Tallon M, Tancredi T, Temussi PA. Conformation–activity relationship of sweet molecules. Comparison of aspartame and naphthimidazolesulfonic acids. *J. Med. Chem.* 1990; **33**: 514–520.
82 Tomchik SM, Berg S, Kim JW, Chaudhari N, Roper SD. Breadth of tuning and taste coding in mammalian taste buds. *J. Neurosci.* 2007; **27**: 10840–10848.
83 Koradi R, Billeter M, Wüthrich K. MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graph.* 1996; **14**: 29–32.

 Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.