Structure-Function Relationships in Sweet-Tasting Proteins

JANE CALDWELL[‡] AND JOHN L. MARKLEY[‡,§]
Graduate Program in Biophysics and [§]Department of Biochemistry,
University of Wisconsin-Madison, Madison, WI 53706.

Summary: Six proteins have so far been isolated that, to the higher primates, have an intensely sweet taste: monellin, thaumatin, brazzein, pentadin, mabinlin, and curculin. Curculin also acts as a taste-modifying protein, as does the protein miraculin. These proteins are all isolated from plants, but span a wide range of molecular weights and biochemical properties. Sequence comparisons show little similarity between the sweet proteins, and suggest that they evolved independently of each other and are descendants of other proteins which were originally parts of the molecular machinery of the plant. Structures have been determined for several of the sweet proteins, but these structures bear no resemblance to each other. Various methods have been used to identify residues in these proteins that are responsible for their sweet taste. Thus far no common motif for a sweet determinant has been identified, although some immunological studies suggest that such a common structure may exist.

Introduction

Although protein is both a significant and necessary component of the human diet, proteins generally are not perceived as having taste qualities. Some exceptions can be found among the smaller peptides, such as the dipeptide sweetener aspartame [1] or the taste-enhancing savory peptide from beef [2], but large polypeptides are generally devoid of taste. In 1972 two significant exceptions to this rule were reported: the proteins monellin [3] and thaumatin [4] were isolated and shown to taste many thousand times sweeter than sucrose on a molar basis. These two proteins were the founding members of a growing family of sweet-tasting proteins, including monellin, thaumatin, mabinlin, pentadin, brazzein, and curculin. Curculin has another unusual taste-active property as well; after its own sweet taste has faded, it still imparts a strong sweet taste to acidic solutions or to water [5]. Like curculin, miraculin is also a "sweet taste-inducing" protein in the presence of acid, although by itself it is flavorless. A sweet taste-suppressing protein, gurmarin, has also been identified, but it is active only in mice and rats [6-9].

This "family" of sweet tasting and sweetness-inducing proteins is really a function-based grouping of structurally and evolutionarily unrelated proteins. Their shared characteristic is their intense sweetness, which is perceived only by humans and Old World primates [10,11], but not to most other mammals including hedgehogs [10], guinea pigs, rats [12], rabbits, dogs, pigs, and hamsters [13]. Their tastes are typically several hundred to several thousand times sweeter than sucrose on a weight basis, with a slower onset, longer duration, and slower decline of the sweet taste response than sucrose [14,15].

All of these proteins are postulated to interact with a sweet taste receptor located on the tongue. Electron microscopic studies have demonstrated the binding of gold-labeled derivatives of monellin and thaumatin to various locations in and around the taste pore in primate foliate taste tissue [16,17]. The receptor is most likely proteinaceous itself because it is destroyed by boiling [18]. It is believed to be coupled to gustducin [19] or a related G-protein which causes a second messenger cascade of cyclic nucleotides [20], suggesting that it is a protein with 7 helical transmembrane segments, by analogy to other G-protein coupled receptor proteins [21-24]. Little else is known about the receptor as it has not yet been isolated, sequenced, or cloned, and it is not known whether it is the only one or merely one of a number of receptors for sweet substances. Some electrophysiological studies suggest the existence of a common receptor for both sweet proteins and smaller sweet molecules, since signals from monellin and thaumatin show cross adaptation with sucrose and various low molecular weight sweeteners as well as with each other [18]. Other studies, however, indicate that there may be multiple receptors with different specificities for sucrose and the sweet

proteins, since taste responses elicited by monellin and thaumatin are more intense at sites along the edges of the tongue, whereas sucrose gives its most intense response at the tip of the tongue [15]. It is generally hoped that studies of sweet proteins will aid our understanding of the sweet receptor, both as high affinity, easily manipulated probes for the receptor itself, and by providing indirect information about the structure of the sweetener-binding pocket.

Sweet Tasting Proteins and Their Origins

Sweet tasting proteins have been found in a variety of unrelated plants spread over two continents. The proteins cover a wide range of biochemical properties:

Monellin was isolated from the "serendipity berry" of *Dioscoreophyllum cumminsii*, found in West Africa (Ghana). Although when it was first isolated in 1969 [25] it was initially thought not to be a protein, its true identity became clear in further studies at the Monell Chemical Senses Center, from which it takes its name [3]. The protein tastes 2,000-2,500 times sweeter than sucrose on a weight basis [26], except in the presence of phosphate buffers which reversibly destroy the sweet taste [25] Monellin is composed of two chains, A and B [27-29], which separately do not taste sweet [28]. The net molecular weight of the AB dimer is 11,000 [26]. Several novel versions of monellin have been created [30,31] in which these two chains have been fused. These engineered "single chain" monellins preserve the sweet taste property of the dimer, but are much more stable [30-32].

Thaumatin was purified from the "katemfe" fruit [33] of *Thaumatococcus danielli*, a rainforest shrub of West Africa. The fruit was traditionally used in this region to sweeten acidified bread, bad palm wine, and underripe fruit [33]. As with many other sweet proteins, thaumatin derives its name from the Latin nomenclature of its source plant, in this case *Thaumatococcus danielli*. Five variants from five different genes [34], have been identified subsequent to the isolation of the two dominant forms, thaumatins I and II [4]. Each of the variants consists of a single 22 kDa polypeptide [35], and the dominant forms are relatively thermostable up to 75 °C without loss of sweetness [36]. Thaumatin tastes 1,600 times sweeter than sugar on a weight basis [4] with a licorice-like aftertaste. Efforts have been directed at over-expressing thaumatin in various transgenic hosts, including *Escherichia coli* and yeast [37-40], thanks to the extensive interest the protein has created for both basic research and commercial markets. It is produced for commercial use by Tate and Lyle, and is marketed under the trade name Talin® for use as both a sweetener and flavor enhancer [41]. Various safety tests demonstrated that thaumatin is fully digestible, has no significant mutagenic or teratogenic effects, and elicits only mild allergenic responses [42].

Mabinlin was extracted from *Capparis masaikai*, which grows in subtropical Yunnan [43]. The protein is found in the seeds, which are chewed for sweetness or medicinal effects and are known as the traditional Chinese herb "mabinlang", from which the protein takes its name [43]. The protein exists in 5 isoforms [43], each of which is 10 times sweeter than sucrose on a weight basis [43,44], and consists of a 12.4 kD disulfide-bonded heterodimer [43]. The 72-residue B chain contains two intrachain disulfide bonds, and is covalently linked to the 33 residue A chain by two additional interchain disulfides [45]. This extensive disulfide cross-linking may be responsible for the thermostability of the mabinlins, which are stable (by both circular dichroism and measures of sweetness) at 80°C for at least two days, with the exception of mabinlin I-1, which denatures after one hour [44].

Both pentadin [46] and brazzein [47] were isolated from the fruit of the climbing vine, *Pentadiplandra brazzeana*, found in western coastal Africa. Unlike brazzein, pentadin was extracted from heat-dried fruit, and the resulting protein has a molecular weight roughly twice that of brazzein [46]. Brazzein and pentadin, respectively, taste 2,000 and 500 times sweeter than sucrose on a weight basis [46,47]. Because the two proteins have roughly similar amino acid compositions, however, it has been suggested that pentadin is a heat-induced brazzein dimer. Brazzein consists of a single peptide chain of 6 kDa [47], which has an acidic isoelectric point [48] and is highly soluble [47]. It is internally crosslinked by 4 disulfide bridges [49], which may contribute to its stability at 80°C for up to four hours [47,48].

Curculin is derived from the pulp of the fruit of *Curculigo latifolia*, a stemless herb that grows wild in Western Malaysia [5]. Its taste is 400-500 times that of sucrose on a weight basis, except in the presence of concentrated salts, particularly divalent cations, which reduce or suppress its sweetness [5,50]. It is a homodimer of a 12 kD polypeptide [5] which contains 2 interchain disulfide bonds [51]. Its taste is stable up to 50°C, between pH 3 and 11 [50]. It also has taste-modifying properties and will confer a sweet taste to either acid or water consumed after the initial taste has faded. This response varies with the type of acid present and seems related to the sourness, rather than the pH, of the acidic solution [50].

Miraculin is extracted from the red berries of *Synsepalum dulcificum*, a tropical shrub found in western Africa [52], where it has been used for generations to sweeten foods [53]. Like curculin, miraculin imparts a sweet taste to acidic solutions or foods, but unlike the other taste-active proteins discussed so far, miraculin has no taste in and of itself [52]. This sweetening property has a slower onset than the response to acids (*i.e.*, a solution of miraculin and citric acid initially tastes sour), but lasts for hours after consuming miraculin [54]. It is a tetramer of a 28 kD polypeptide [51] and is a glycoprotein [52,54]. Each polypeptide subunit contains one intrachain disulfide bond and is covalently associated into 44 kD dimers by three interchain disulfide bonds [51]. Like curculin, miraculin imparts sweetness to all acids tested so far, and the resulting sweetness is related to the sourness of the acid, rather than the pH of the solution [54]. Electrophysiological studies showed that the sweet taste of miraculin is merely superimposed upon the pre-existing sour taste, and does not actually eliminate the acidic nerve response [54].

Sequence Comparisons

Comparisons of the amino acid sequences of the sweet-tasting and sweetness-inducing proteins have failed to reveal a "consensus sequence" for sweet taste, or any indication of an evolutionary relationship. Rather than sharing a common ancestor, it seems more likely that each protein evolved independently from pre-existing non-sweet biological components in the parent plant. This hypothesis is supported so far by the resemblance between each of the sweet proteins and a variety of non-sweet plant proteins. It is interesting to note that in most cases, these non-sweet proteins have defense-related roles, such as protease-inhibitors or fungicides. This commonality is most likely coincidental, however, and should not be surprising: the stationary nature of plants renders them vulnerable to wounding, predators, or parasites; it is to be expected that much of their internal machinery is devoted to defense.

Monellin also is highly similar to the cystatin thiol protease inhibitors, which are not perceived by humans as sweet. Monellin shares approximately 20% sequence identity with the rice cystatins oryzacystatin I & II [55]. If the A and B chains in monellin were joined, the cleavage site between them would correspond exactly to the highly conserved binding site of the cystatins. Its structure closely resembles those of chicken cystatin and the cystatin-like protein human stefin B: the backbone coordinates of the structures of each show approximately 2 Å rmsd values with those of monellin [55]. Some similarity has also been noted between the N-terminal region of the A chain of monellin and the eight-residue savory-tasting beef peptide [56], however this resemblance is limited to only four residues and has yet to be shown to be functionally significant.

The thaumatins have been shown to be homologous to a remarkable number of both acidic and basic proteins found in tomato [57], potato [58], tobacco [59-63], maize [64-66], soybean [67], wheat [68], and barley [69]. Some are produced under relatively normal conditions, *e.g.*, floral differentiation in tobacco [59] or maturation of potato plants in a greenhouse [58], while others are induced by pathogens and/or environmental stresses (including salt stress, wounding, chemical pollution, or UV light). These include the pathogen-response (PR) proteins from tobacco, some of which also act as osmotins or fungicides [60], the maize fungicides zeamatin [60] and maize antifungal protein, which is 99% identical [65] to the maize bifunctional inhibitor of trypsin and alpha-amylase [66], as well as the tomato osmotin NP24/P23 which is also induced by viruses [57]. These proteins most likely share the same three-dimensional fold as thaumatin, since the positioning of their cysteines is conserved

[67]. Given this apparent evolutionary link between thaumatin and some ancestral defense protein, it is interesting to note that thaumatin itself has weak antifungal activity [70]. Interestingly, most of the residues implicated in the sweet taste of thaumatin are not conserved among the thaumatin-like proteins [68].

Curculin shares 41% of its sequence with *Galanthus nivalis* (snowdrop) agglutinin (GNA), a mannose-binding lectin in which 2 of the 4 cysteines have been conserved. Although most of the residues involved in the GNA mannose-binding pocket are also conserved in curculin, structural modeling studies suggest that this pocket will be non-functional in curculin [71]. Interestingly, the plants that produce curculin and GNA are related, both belonging to the taxonomic order *Asparagales*, which supports the idea of a common ancestor for the two proteins. Coincidentally, as with the thaumatin-like proteins, GNA has been suggested to play a role in defense and was shown to enhance the resistance of transgenic tobacco to insects [72].

The mabinlins have 25-46% amino acid sequence identity with 2S seed storage proteins found in *Arabidopsis thaliana* (mouse ear cress), *Brassica napus* (rape), *Ricinus communis* (castor bean), and *Bertholletia excelsa* (Brazil nut) [45,73]. The pattern of cysteines is conserved among these proteins [43,45], suggesting a common structure. Analysis of the sequences of the precursor proteins shows that they may also undergo similar post-translational processing routes, as they have similar predicted cleavage sites and signal peptides [73].

Brazzein exhibits a fold common to the antifungal gamma thionins [74] from barley and wheat [75]. This fold, interestingly, is similar to that found in various scorpion neurotoxins and insect antibiotics and fungicides, although the limited sequence similarity (approximately 20% identity) is accounted for almost entirely by the structurally required cysteine residues. These cysteines occur in a very rigidly spaced pattern, identified originally in scorpion neurotoxins and insect antibiotics and fungicides [76], and gives rise to a well-conserved "cysteine-stabilized alpha-beta fold," consisting of a helix bonded through two disulfide bridges to a two or three stranded beta sheet [77]. A similar pattern of cysteines is found in a variety of plant proteins, including pathogen-induced gamma-thionin-like proteins as well as inhibitors of insect alpha amylases, although the sequence similarity is low, ranging between 17 and 24% (Figure 1). This cysteine motif also occurs in a family of bifunctional trypsin/chymotrypsin inhibitors [78,79], which share a slightly higher (30%) sequence identity with brazzein, although it lacks the reactive site residues [75] and is devoid of proteinase inhibitor activity [48]. Thus it seems likely that, like thaumatin, brazzein evolved from an ancestral plant defense protein, perhaps a proteinase inhibitor or fungicide.

Miraculin shows 36-50% sequence identity at its amino- and carboxyl-terminal residues to the soybean Kunitz-type trypsin inhibitors A and C [51]. It shows no extensive sequence similarity to any of the sweet-tasting proteins, although six tripeptides in its sequence also appear in curculin [5,51] and are believed to be solvent exposed in the latter [71].

The sequences of the sweet-tasting and sweetness-inducing proteins have been compared to each other in efforts to identify any existing "consensus sequence" for sweet taste. As a result, several tripeptides have been identified that are found in the amino acid sequences of both monellin and thaumatin [35], monellin and curculin [5,51], thaumatin and curculin [5,51], mabinlin and curculin [51], and miraculin and curculin [51]. One possibility is that some subset of these common tripeptides could cluster to form a structurally similar sweet determinant in each protein. In practice, however, these tripeptides have yet to be shown to be involved in sweet taste. Their role seems somewhat dubious, given that the tripeptides shared by monellin and thaumatin do not overlap with any of those shared by either monellin and curculin or thaumatin and curculin, and none of these peptides are found in the sequence of brazzein. Furthermore, once the structures of monellin and thaumatin were elucidated, their shared tripeptides were examined more closely. Of those five tripeptides, only three were conformationally similar, only two of those were solvent-accessible, and the spatial separation of those two differed by so much as to render their participation in a common sweet determinant virtually impossible [80]. Beyond these tripeptides, there is no significant sequence similarity between these sweet and sweetness-inducing proteins.

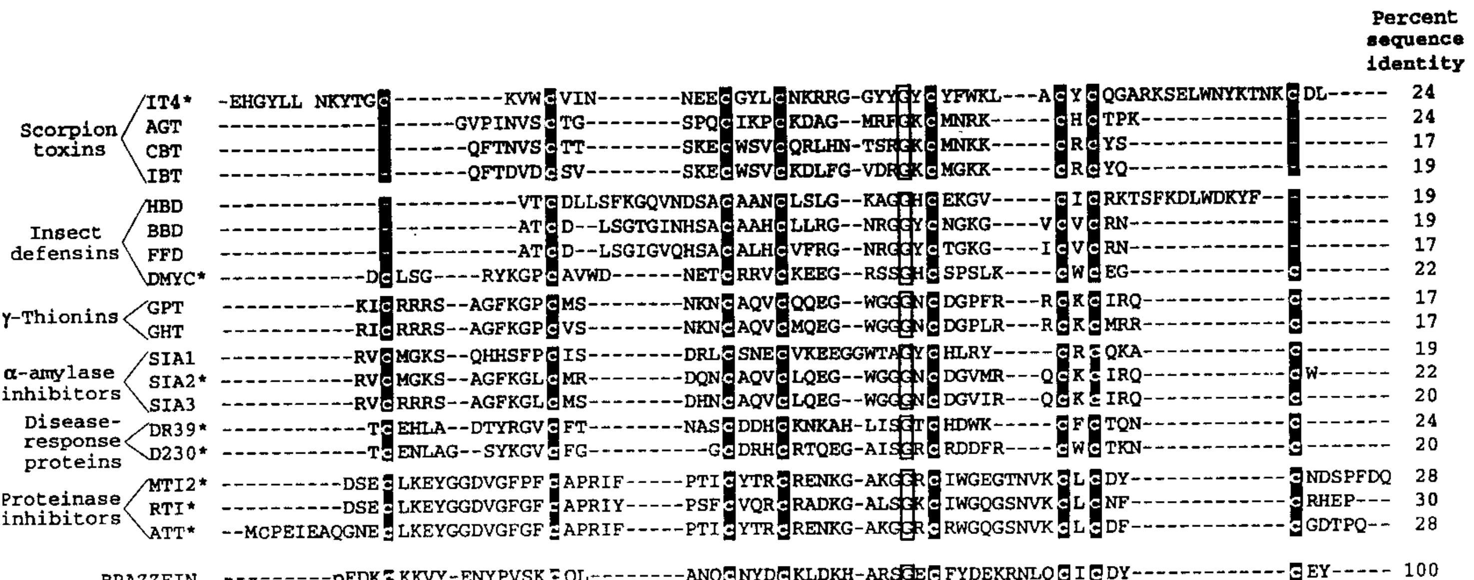

Fig. 1: Amino acid sequence alignments of brazzein with representatives from each class of protein that contains the CSαβ consensus motif. White and dark boxes indicate, respectively, the conserved glycine and cysteine residues. Lines at the bottom of the figure indicate known disulfide pairings; proteins for which pairings have not been determined are marked by asterisks. The percentages of residues in the sequence of brazzein (PIR accession number S51208) that are identical to those in each aligned sequence (% identity) are indicated. Percent sequence identities were determined after aligning the glycine and cysteine residues of each individual sequence with those of brazzein using MSA version 2.1 [113,114]. Protein identifiers (in parentheses) are SWISS-PROT accession numbers except where otherwise indicated. IT4, *Androctonus australis* Hector anti-insect toxin 4 (P21150); AGT, agitoxin 2 (P46111); CBT, charybdotoxin (P13487); IBT, iberotoxin (P24663); HBD, honeybee defensin (P17722); BBD, black blowfly defensin (P10891); FFD, flesh-fly defensin against gram positive bacteria (P3150); DMYC, fungicide from *Drosophila melanogaster* (P41964); GPT, wheat γ1-purothionin (P20158); GHT, barley γ1-hordothionin (P20230); SIA1, SIA2, and SIA3, respectively, sorghum α-amylase inhibitors 1, 2, and 3, (P21923, P21924, P21925); DR39 and DR230, fungally-induced garden pea disease-resistance response proteins (Q01784, Q01783); MTI2 and RTI, inhibitors of trypsin and chymotrypsin from white mustard (P26780) and oil-rape seed (P80301); ATT, *Arabidopsis thaliana* putative trypsin inhibitor (GenBank Z46816).

Three-dimensional structures

The lack of any compelling sequence basis for sweet taste suggests that it is necessary to seek sweet determinants in the tertiary, rather than primary, structural features of these proteins. Sequence analyses have failed to locate a consensus "sweet" sequence, and moreover, attempts to isolate sweet tasting peptides from these proteins have so far been unsuccessful [81]. Disruption of the three dimensional structures of the sweet proteins, however, whether by heat [4,50], chemical denaturants [82,83], or alkylation of structurally important disulfides [4,26,84], generally destroys their sweet taste. Studies of monellin have correlated its loss of sweetness with changes in the three-dimensional structure monitored by circular dichroism [83], fluorescence, or light scattering [85]. Similar results were obtained from circular dichroism and proton magnetic resonance studies of thaumatin [36], and circular dichroism studies of mabinlins I-1, II, III, and IV [44]. Thus it seems that the chemical moieties involved in the sweet determinant only achieve the required configuration in the native three dimensional structure.

Structural investigations have been performed or are underway on most of the sweet proteins. Ideally, these structural studies will eventually include complexes between sweet-tasting proteins and the (as yet unidentified) sweet taste receptor protein.

The structures of both monellin [32,86,87] and its engineered single-chain counterpart [32,88] have been determined by x-ray crystallography, to final resolutions of 2.75 and 1.7 Å, respectively, and have been confirmed by solution-state NMR spectroscopy [87,88]. The structures, which are essentially identical, contain a five-strand beta sheet with a right-handed twist, whose concave side is filled by a 17 residue alpha helix. The beta sheet has bends or bulges in the second and fifth beta strands. The A and B chains each contribute several strands to the beta sheet and are intimately associated; unlike many other heterodimers, the two chains do not delineate easily separable domains or subunits. It is interesting to note that the N- and C-termini of the B and A chains, respectively, are near each other in the structure. This close association of the two chains correlates nicely with the observed structural and amino acid sequence similarity to the single-chain cystatins, as discussed previously.

Two independent crystallographic studies have yielded virtually identical high resolution structures of thaumatin with resolutions of 1.65 [89,90] and 2.6 Å [91]. The structure is composed mostly of antiparallel beta-sheet arranged in a flattened beta barrel with a right-handed twist. This central domain is attached by disulfide bonds to two smaller domains which are composed, respectively, of two beta strands connected by an omega loop, or of one alpha helix bordered by three short helical fragments. No structural similarities between thaumatin and monellin have yet been reported, and their global folds are unrelated.

The structure of brazzein has been determined at low resolution by NMR to approximately 2 Å rmsd versus the mean structure [75,92], and crystals for x-ray studies have also been obtained[93]. The structure contains a short three-stranded beta sheet which is disulfide bonded to two short alpha helices. As discussed above, the overall fold resembles that of the barley and wheat gamma thionins (Figure 2), but not monellin or thaumatin (Figure 3). Refinement of the NMR structure is ongoing.

Curculin crystals have been isolated that diffract to at least 3 Å [94], although an x-ray diffraction structure has not yet been published. A three-dimensional model, however, has been calculated [71], based on the amino acid sequence resemblance between curculin and GNA (as discussed above), whose structure is known. This model does not resemble the global structures of monellin, thaumatin, or brazzein [71,75,92].

The Search for a Sweet Determinant

The two most pressing questions concerning the sweet proteins are (1) the composition and structures of the individual sweet determinants and (2) the possible existence of a common sweet determinant. The first question has been investigated to varying degrees for each protein by chemical, synthetic, and molecular biological routes. In the end, both of these questions will probably be answered by a careful combination of site-directed mutagenesis and structure determination.

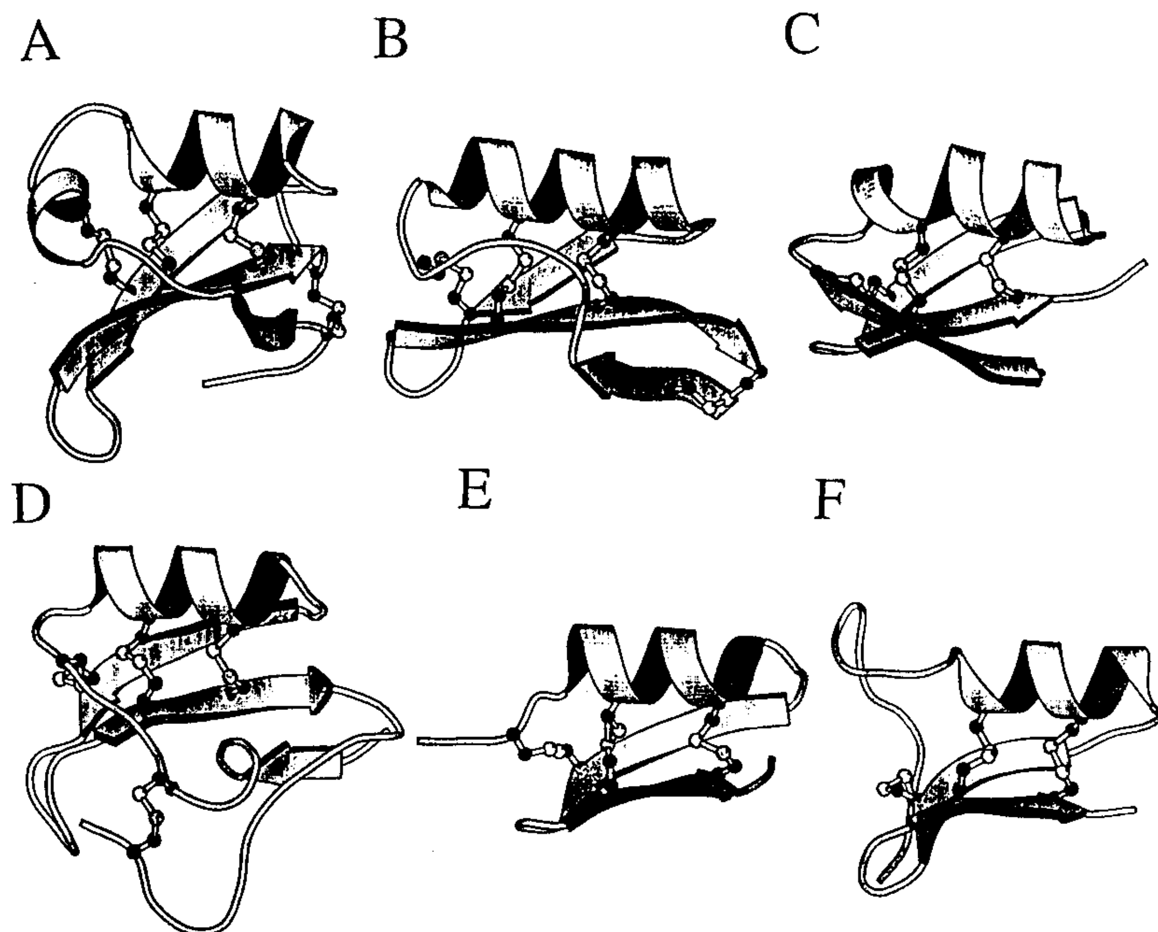

Fig. 2: Schematic ribbon drawings of brazzein and its structural homologues. Ball-and-stick figures indicate disulfide bonds. (A) Brazzein [75], (B) barley γ_1-hordothionin [74] (PDB accession code 1GPT), (C) agitoxin 2 [115] (PDB accession code 1AGT), (D) scorpion toxin II [116] (PDB accession code 1PTX), (E) toxin PO_5-NH_2 [117] (PDB accession code 1PNH), and (F) insect defensin A [77] (PDB accession code 1ICA). Drawings were produced with Mol Script [118].

Examination of Sweet Protein Variants

Investigations of the sweet determinant of monellin have been particularly extensive, and several excellent reviews on the subject have been published [95,96]. Early chemical modification studies indicated the importance of lysine [97] and cysteine [98] residues in monellin. Although some of these studies indicated that the cysteine residue was probably buried [98,99], a variety of other studies were performed to investigate its role in the sweet taste determinant 28,32,100. Only later did the x-ray structure [32] conclusively demonstrate that this residue is solvent inaccessible and therefore unlikely to be involved in the sweet determinant. Comparison of these early studies with those initiated after the three-dimensional structure of monellin was determined illustrates the usefulness of structures in efficiently guiding mutagenesis experiments. Monellin variants have been produced more recently by both synthetic and molecular biological means [95,96,101-103], and these studies so far implicate primarily residues 6 through 9 of the B chain, and to a lesser extent, residues 20, 22, and 36 of the A chain [96], none of which, interestingly, are lysines. These residues form two clusters on the protein

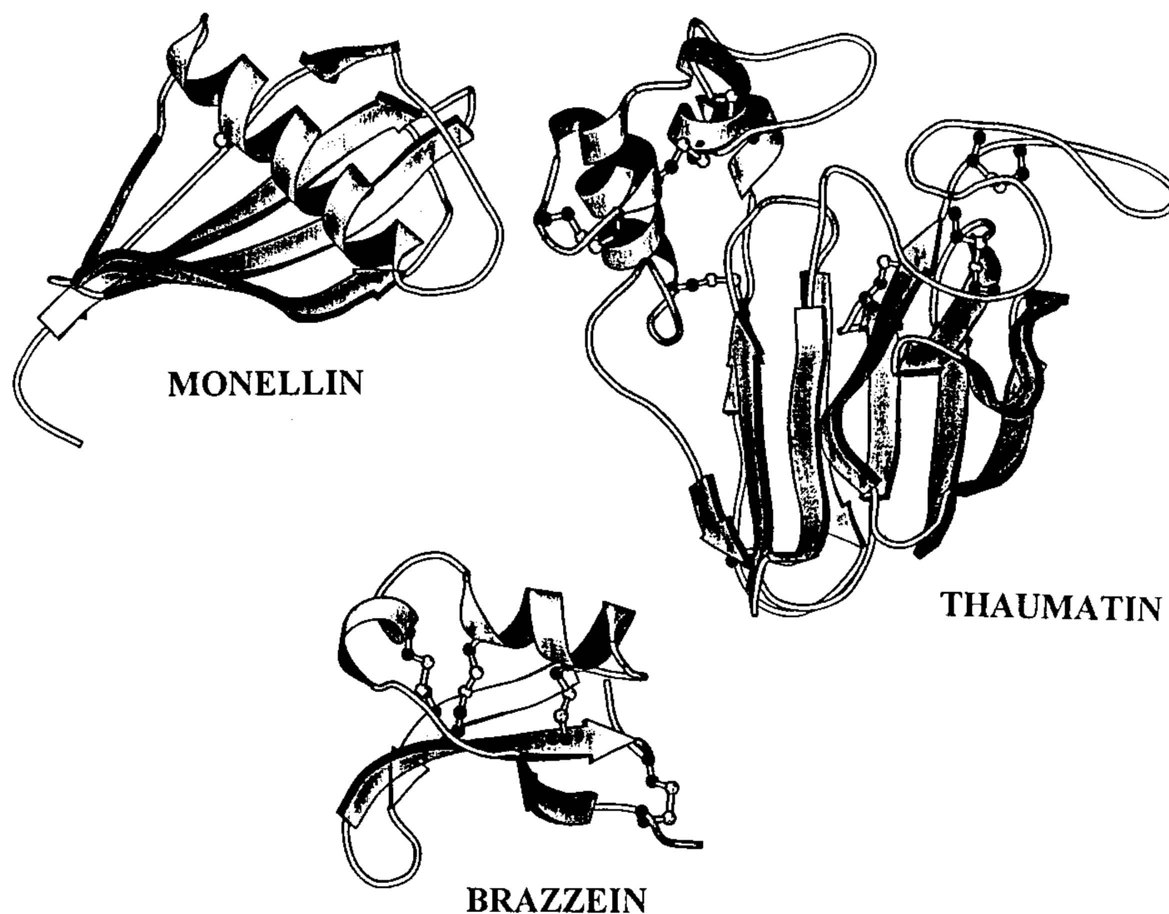

Fig. 3: Structures of monellin [80] (PDB accession code 3MON), thaumatin [91] (PDB accession code 1THW), and brazzein [75]. Drawings were produced with MolScript [118].

surface located 11-25 Å apart. It is not yet known whether the residues in both of these sites are involved in binding to the sweet taste receptor, *e.g.* by forming an extensive surface of interaction, whether these two clusters represent two distinct binding sites, or whether some of the mutations induce conformational changes in the protein.

Chemical modification studies of thaumatin indicated that the sweetness of the protein is decreased gradually by progressive acetylation of its lysines [104]. Methylation of these same residues, however, had no effect on sweetness, or for that matter on its isoelectric point [104]. Taken together, these results suggest that the charge of the lysines might be important for sweetness. In another study, amidation and subsequent methylation of the carboxylates of aspartate and glutamate were found to alter the sweetness as well as the electrophysiological properties of the protein [105]. Other studies have implicated an aspartic acid at position 113 as being essential to properly fold the protein into a sweet form [39]. Study of the three-dimensional structure of thaumatin suggested that residues 19-29 might be important, since these residues are not present in the non-sweet thaumatin-like plant defense proteins [106], and because residue 25 adopts an energetically unfavorable conformation [91]. Residues 77-84, which are recognized by a polyclonal anti-thaumatin serum that cross-reacts with monellin, may also be critical. It was suggested that these residues might form an aspartate-like site with residues 19-29, which are also recognized by monoclonal anti-thaumatin antibodies that cross-

react with monellin [106]. A clear picture of the sweet determinant for thaumatin has yet to emerge from these clues, and careful site-directed mutagenesis studies will probably be necessary for further progress.

Various chemically modified brazzein samples have been produced and tested for sweetness [48]. Modification of arginine, histidine, lysine, or tyrosine residues was found to destroy the sweet taste. These residues, which may be involved in the sweet determinant, are being investigated further by site-directed mutagenesis:an overexpression system for brazzein has been developed [107], and is poised to produce brazzein variants.

Immunological Studies

Thus far, each of the sweet proteins has been shown to have a three-dimensional structure which differs drastically from those of the other sweet proteins (Figure 3), and no common structural feature which might serve as a common sweet determinant has been identified. Immunological studies, however, suggest that such a common structural feature may exist. Both polyclonal antisera and monoclonal antibodies have been obtained which cross react between the sweet proteins, as summarized in Table 1 [5, 31, 51, 80, 89,106, 108-112]. Immunological cross-reactivity seems to place monellin and thaumatin in a class together, and miraculin, curculin, brazzein, and mabinlin in a separate class. A tempting conclusion suggested by these results is that the common antigenic determinant in each case may also be the sweet determinant. Such an interpretation seems to be supported by studies in which anti-thaumatin polyclonal antiserum cross-reacted with monellin, but not with iodinated monellin, which is not sweet [108]. Additional support for this hypothesis includes the observation that low molecular weight sweeteners, including cyclamate and aspartame, compete with thaumatin for binding to an anti-thaumatin polyclonal antiserum, and that the degree of competition correlates well with the degree of sweetness [109]. The observation that some anti-body:sweet-protein complexes no longer taste sweet [80], suggests that both the antigenic and sweet-taste determinants share common or overlapping sites. Given the large size of an antibody, however, its steric hindrance effects on other binding interactions (*e.g.* to the taste receptor) would probably be appreciable.

Results from studies which utilize polyclonal antisera must be interpreted with care, however, since a polyclonal serum contains a mixture of antibodies that individually recognize different antigenic features with different affinities. This point is illustrated well by studies in which cross-reacting antisera were used to identify "related" peptides in monellin and thaumatin[31]. Closer examination of the peptides revealed that they each contained a tyrosine and an aspartic acid residue, which adopted aspartame-like conformations in the three-dimensional structure of each protein. Contrary to expectations, however, mutation of these residues in monellin did not destroy the sweet-taste activity: instead, substitution of a glycine for the tyrosine had no effect [95], and changing the aspartate to an asparagine rendered the protein even sweeter [103]. It seems that either the use of polyclonal antisera gave misleading results, or that the common antigenic determinants are unrelated to the sweet determinants.

A similar caveat is raised by a study[110] that utilized several monoclonal antibodies. In that study, monoclonal antibodies against both monellin and thaumatin were evaluated for their ability to bind thaumatin. The amount of bound thaumatin was

Table 1. Serological Cross-Reactions between Sweet Proteins*

	monellin	thaumatin	curculin	mabinlin	miraculin	brazzein
monellin	X	X(106,109,110)	0 (51)		0 (51)	
thaumatin	X(80,110,112)	X	0 (51)		0 (51)	
curculin			X		0(5), X(48,51)	X (48)
mabinlin			0 (111)	X		X (48)
miraculin			X (51,111)		X	X (48)
brazzein						X

*The protein to which each antiserum (monoclonal or polyclonal) was generated appears at the top of each column; proteins tested for cross-reaction appear in rows. Numbers in parentheses indicate the references cited. Abbreviations: "0" indicates no cross reaction was observed, "X" indicates cross-reaction was observed, and blank spaces indicate combinations which were not tested.

quantified for each antibody alone and in the presence of each of the other antibodies. The degree of overlap, if any, between their antigenic determinants was estimated by observing interference between the antibodies. These estimates revealed that those anti-thaumatin antibodies that cross-reacted with monellin recognized a different, non-overlapping epitope on thaumatin than that recognized by cross-reacting antibodies raised against monellin. The finding that monellin and thaumatin thus may share more than one antigenic determinant suggests that common antigenic determinants are not as rare as might be hoped, and that serological similarity does not necessarily imply a common functional mechanism. It is still possible, however, that one or both of these common antigenic determinants is also a sweet determinant. In any case, this study implies that the interpretation of serological cross-reactions is not simple, and that more detailed immunological studies should be performed.

Summary and Future Prospects

Only six proteins that taste sweet to higher primates—monellin, thaumatin, pentadin, curculin, mabinlin, and brazzein—have so far been isolated and characterized. A seventh protein, miraculin, acts as a sweet-taste inducing protein, as does curculin. Although our knowledge of the biochemical properties, sequences, and structures of these proteins is extensive and still expanding, the nature and function of their sweet determinants is still a mystery, and the receptor(s) to which they bind are still unknown. It remains to be seen whether all sweet proteins act on the same receptor or exhibit similar modes of receptor binding, and whether their binding sites are related to those of lower molecular weight sweeteners.

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