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Chemostimulatory Protein: A New Type of Taste Stimulus

Two proteins that taste sweet are now known.

Robert H. Cagan

A wide range of biological activities is known to be effected by various proteins, including enzymic catalysis, membrane transport, immunological specificity, hormonal activity, and maintenance of structural integrity. Until very recently, however, no macromolecule was known to act as a specific taste stimulus in man. Solms in 1969 (*1*) correctly summarized the earlier state of knowledge: "It is generally accepted that proteins exert no taste activity." In addition to the well-known sweet taste of many sugars, certain D-amino acids were known to possess some sweetness although their L-enantiomers were usually tasteless or bitter (*1, 2*). Recently, the synthetic dipeptide ester L-Asp-L-Phe-Me (*3*) and certain closely related dipeptide esters, as well as several other α-amides of L-aspartic acid, were shown to be sweet (*4*). Other small peptides, however, were known to be bitter, sour, or tasteless (*5*). Of the molecules previously known that evoke specific taste sensations, such as sweet, sour, salty, and bitter, none have been proteins. In this article I review recent discoveries of three taste-active proteins of plant origin, and propose that two of these proteins should be called "chemostimulatory proteins" because of their sensory effect. The third protein was originally called both the "taste-modifying protein" and "miraculin" (*6, 7*). "Taste-modifier protein" is used here as a generic term, and the specific designation "miraculin" is retained for the single known protein of this type.

Both of the known chemostimulatory proteins are intensely sweet. The single known taste-modifier protein changes the normal taste sensation of acids from sour to sweet after the tongue has been treated with the protein. Chemostimulatory and taste-modifier proteins are to be distinguished from receptor proteins. The latter are cellular proteins, often in the plasma membranes of the receptor cells, that interact with extracellular chemicals. Examples of receptor proteins are the acetylcholine-receptor proteins of neural tissues (*8*), the insulin-receptor proteins of liver and fat cells (*9*), and the galactose-binding protein that is essential for chemotaxis of the bacterium *Escherichia coli* toward galactose (*10*). On the other hand, receptor proteins have not been isolated from taste receptor cells (*11*) although their existence has often been postulated in the past.

Three Taste-Active Proteins

The botanical origins, geographical distributions, and biochemical properties of the three proteins (miraculin, monellin, and thaumatin) now known to have defined effects on the taste system of humans are summarized in Tables 1 to 3. Perhaps the most extraordinary of the three proteins from a phenomenological point of view is miraculin, the taste-modifier protein. After the tongue is treated with this glycoprotein, acids which are normally sour then taste sweet. The effect of the drug is prolonged; for example, the effect lasted for more than 3 hours after a 2.3 micromolar solution of miraculin was held in the mouth for 5 minutes (*12*). Miraculin does not itself taste sweet. The fruit and its effect have been known for many years (*13*) and the isolation of the glycoprotein was reported in 1968 independently by two laboratories (*6, 7*). It has substantial potential as an experimental tool in taste research.

Monellin was isolated in electrophoretically pure form and characterized as a sweet-tasting protein (*14*); almost simultaneously thaumatin was partially purified and reported to be a sweet-tasting protein (*15*), confirming independently that a protein can act as a specific taste stimulus. The finding that monellin is a protein was not in agreement with an earlier conclusion (*16*) that the sweet principle in *Dioscoreophyllum cumminsii* is a carbohydrate; the conclusion that it is a protein has been confirmed, however, by recent independent findings (*17, 18*). Because of the intensity and persistence of the sweet taste of monellin, this protein holds considerable promise as an experimental tool in taste research.

Chemistry of Miraculin, Monellin, and Thaumatin

Unlike miraculin, neither monellin nor thaumatin are glycoproteins. The molecular weights of the chemostimulatory proteins are considerably lower than that of miraculin (Table 2) (*19*). Active monellin consists of a single polypeptide chain of molecular weight 10,700 (*20*). The size of the active thaumatin molecule is not known with

The author is a member of the Monell Chemical Senses Center, University of Pennsylvania, a research scientist at the Veterans Administration Hospital, Philadelphia, and assistant professor of biochemistry, School of Medicine, University of Pennsylvania, Philadelphia 19104.

Table 1. Latin and common names of plants or fruits from which miraculin, monellin, and thaumatin are obtained, and geographical distributions of plants (*13, 24*).

Item	Miraculin	Monellin	Thaumatin
		Botanical origin	
Latin	*Synsepalum dulcificum* (Schum. & Thonn.) Daniell, Family Sapotaceae	*Dioscoreophyllum cumminsii* (Stapf) Diels, Family Menispermaceae	*Thaumatococcus daniellii* Benth., Family Marantaceae
English	Miracle fruit, miraculous berry, miraculous fruit, sweet berry	Wild red berry, guinea potato, serendipity berry	Miraculous fruit, miraculous berry
Yoruba	Agbayun	Ito-igbin, ayun-ita	Katemfe, kete-nfe, kekerenfe
Twi	Asaa, asewa	Moframofratwe	Anwuram-asie, owuramsie
Ewe	Ledidi		Alklamakpa (also used for *Culcasia* sp.)
Ga-Adanme	Taami-tso, tamami, tamami-tso, tãami, tamlami, tama		Agidibaa
Wassaw	Abrewe		
Fante	Abrewe, asarba, asaba		
Krobo	Tamlami, tamami-tsho		
Timne		Ekali-bonte, kaligbonde (as for Rhigiocarya)	
Ibo		Nmimimi nwambe	
Ashanti			Anworam, owuram
Bakoué			Urugua méremné
Néyau			Bobo abi
Bété			Bogridjia, bobruidja
Tivi			Ijowol
Efik			Ninkon
		Geographic distribution	
	West Africa (Ghana to Congo area), introduced to Puerto Rico and Florida	West Africa (Guinea to Cameroon), Gabon, Zaire, Sudan, Southern Rhodesia, Mozambique	West Africa (Sierra Leone to Congo area), Sudan, Uganda

certainty, but evidence indicates that its molecular weight is about 21,000 (*21, 22*). The limited evidence [there is about one residue of methionine per polypeptide chain with a molecular weight of 21,000 (see Table 3)] is consistent with thaumatin also being a single polypeptide chain. It is possible that the larger miraculin molecule possesses quaternary structure, but this is not known. It was noted that miraculin forms even larger aggregates (80,000 to 1,000,000 molecular weight) under some conditions (*6, 7*). The question of the size of the monomeric thaumatin and miraculin molecules could be answered by comparing the size of the polypeptide chain under native and under "reduced and dissociated" conditions as was done with monellin (*20*).

All three proteins are strongly cationic, with isoelectric points of 8.3 or above (Table 2). Whether this strong cationic character is important in the mechanism of taste receptor stimulation is not known, but it is a feature that warrants further study. Specific chemical modification of lysyl residues under mild conditions is one obvious possibility.

The important question of the precise role of the tertiary structure in taste activity of these proteins has not been answered. The biological activities of the three proteins under certain conditions are labile, and some evidence does suggest that unfolding of the proteins leads to loss of biological activity. Each of the proteins loses activity after being heated, and although they are apparently stable over a wide range of *p*H values, they do become inactivated at extremes of *p*H (below about *p*H 2 and above *p*H 10 to 12) (*6, 7, 14, 15, 18, 20, 21*). Another unanswered question is that of the reversibility of the inactivation by extremes of *p*H. It was reported (*21*) that thaumatin loses its sweet taste after cleavage of the disulfide bridges (*23*); this also implicates the tertiary structure as a determinant of sweetness.

Several aspects of the amino acid compositions (Table 3) are worth noting. When the proportions of various types of amino acids are calculated, interesting similarities among the three

Table 2. Taste effects and biochemical properties of miraculin, monellin, and thaumatin.

Properties	Taste-modifier protein miraculin	Chemostimulatory proteins	
		Monellin	Thaumatin
Taste effect	Prior exposure of tongue to miraculin causes sour substances to taste sweet; activity can persist for several hours (*6, 7, 12*)	Intensely sweet taste; persistent sweet aftertaste (*14*)	Intensely sweet taste; licorice aftertaste (*15, 21*)
Chemical nature	Glycoprotein (6.7 percent carbohydrates: L-arabinose, D-xylose) (*6*) Glycoprotein (7.5 to 21 percent carbohydrates: glucose, ribose, arabinose, galactose, rhamnose) (*7*)	Protein (*14, 17, 18, 20*)	Protein (*15, 21*)
Molecular weight	44,000 (*6, 19*)	10,700 (*20*)	18,000 to 21,000 (*21, 22*)
Isoelectric point	8.3 to 9 (*6, 7*)	9.3 (*20*)	> 11.7 (*15, 21*)
Sweetness relative to sucrose*	None (*6, 7*)	About 3,000 times (weight) or 90,000 times (molar) †	750 to 1,600 times (weight) or 30,000 to 100,000 times (molar) (*15, 21*)

* Threshold determinations. † The values for monellin are only approximate. The original maximum value of 3,000 times more effective than sucrose (*14*) was converted to a molar basis by using the molecular weight of 10,700 for monellin (*20*).

proteins emerge, as shown in Table 4 [see also (*22*)]. More data are required in order to determine whether the similarities extend to the amino acid sequences. From the amino acid compositions (Table 3), it should in principle be possible to explain the high isoelectric points of the proteins. It was pointed out by van der Wel (*15*) and van der Wel and Loeve (*21*) that thaumatin has an excess of basic side chains above the free acidic groups. However, the data were based upon the assumption that the ammonia recovered during standard amino acid analysis arose from the amide groups. Because such values might be high, special attention to actual amide content should be given. From the minimum values of ammonia recovered during standard amino acid analyses of monellin, we concluded (*20*) that a simple excess of total basic groups over acidic groups could not account for the high isoelectric point of monellin. Actual amide content has not yet been determined on this protein either. Data on amide content are lacking for miraculin, although ammonia was determined during amino acid analyses. Based on these data (Table 3) there is an excess of basic above acidic side chains.

The absence of histidine in monellin has been noted (*20*) to provide a useful analytical criterion of purity when this protein is prepared; the same would be true for thaumatin, since it too lacks histidine.

The single methionine residue per polypeptide chain in monellin and possibly in thaumatin (Table 3) suggests that chemical cleavage with cyanogen bromide might be an effective means to study both the structure and taste activity of the chemostimulatory proteins. Whether a smaller sweet-tasting polypeptide fragment can be produced by this means will have to await further experimentation. In addition, limited proteolysis with proteolytic enzymes may be a feasible approach in this work. The question of a sweet polypeptide fragment is, of course, closely related to the question of the role of the tertiary structure in eliciting sweetness.

Botanical Origins

Thaumatococcus daniellii is monocotyledonous, while *Synsepalum dulcificum* and *Dioscoreophyllum cumminsii* are dicotyledonous members of two different families (*13, 24*). There is no information available on the development of the proteins in the berries during plant maturation.

Implications for Biochemical Mechanisms of Taste

Suggestions have been made that the initial interaction of a taste stimulus molecule with a receptor cell occurs on the microvilli (gustatory microvilli) of the taste receptor cells. This hypothesis implicates the plasma membrane as an important locus of specificity and transduction mechanisms. Although the plasma membrane does seem to be a likely location by analogy with certain other receptors (*8, 9*), the evidence to support this hypothesis is largely indirect (*25*). The ability of macromolecules to elicit taste sensations provides important, although indirect, evidence supporting this idea. The mechanism of action of miraculin that was proposed by Kurihara and Beidler (*12*) involved the initial binding of miraculin to the plasma membrane. Subsequent treatment with acid was suggested to induce a conformational change in the membrane, leading to stimulation of the "sweet" site on the membrane by the arabinose and xylose moieties of the bound miraculin. It was suggested that at least certain of the binding sites for miraculin are in close proximity to "sweet" receptor sites. They showed that in the presence of gymnemic acid, which inhibits sweet sensations, miraculin no longer alters the taste of acids; in this case the acids taste as sour as they do normally (*12*). The effect of miraculin therefore seems to be on the taste receptors rather than on the acid in solution [see also (*6*)]. The initial interaction of monellin and of thaumatin is postulated to occur at the plasma membrane of the cell. It is suggested as unlikely that either monellin or thaumatin, which have molecular weights above 10,000, would first pass through the membrane of a receptor cell before initiating depolarization of the receptor cell. Of course, this latter possibility cannot be completely rejected without direct experimental evidence on transport of these as well as other stimulus molecules. Studies of the binding of these three proteins to membranes have not been reported, though this approach would seem to be a promising line of inquiry.

The persistence of the effects of miraculin, monellin, and possibly thaumatin (Table 2) raises the possibility that these proteins are firmly bound to taste receptor cells. Whether the binding of any of the three is specific to only the receptor cells and specific to particular sites on the microvilli remains to be determined (*26*). If the proteins are also bound nonspecifically, this could severely limit their use as specific ligands to assay for the presence of taste receptor sites during biochemical studies.

The persistence of the effects of the proteins raises an important physiological question regarding peripheral adaptation. That peripheral adaptation can occur with salt has been reported. The time for adaptation to salt measured by electrophysiological recordings from the human chorda tympani nerve corresponded well with the adaptation time reported by the subjects (*27*). The persistence of the effect of miraculin for 3 hours or longer (*12*) and the ability of monellin to elicit a longer-lasting sweet taste sensation than other sweet compounds (*14*) were noted above, but these characteristics have not been carefully studied for monellin. The persistence of the effects of

Table 3. Amino acid compositions of taste-modifier and chemostimulatory proteins. The data for each of the three proteins are expressed in numbers that are essentially the numbers of residues per minimum molecular weight of the polypeptide. The data for monellin are residues per molecule (*20*), those for thaumatin are calculated from the data for "thaumatin I" [(*21*); see also (*22*)] and are expressed as moles per 21,000 grams of protein, and those for miraculin are residues per 100 residues [(*6*); see also (*19*)].

Amino acid	Amino acid residues		
	Miraculin	Monellin	Thaumatin
	Nonpolar		
Glycine	9.8	7.8	17.6
Alanine	6.3	3.0	12.3
Valine	8.0	3.7	7.5
Leucine	6.5	5.5	8.0
Isoleucine	4.7	6.1	6.4
Proline	6.0	6.5	10.6
Phenylalanine	5.0	5.0	9.3
Tryptophan		1.0	3.2
Methionine	1.0	0.9	0.8
	Polar, uncharged		
Serine	6.1	1.4	9.8
Threonine	6.1	3.5	15.9
Half-cystine	2.3	0.9	12.0
Tyrosine	3.6	6.7	6.4
	Basic		
Lysine	7.9	8.4	9.2
Arginine	4.7	7.1	10.2
Histidine	1.8	0	0
	Acidic		
Aspartic acid	11.3	10.0	18.0
Glutamic acid	9.2	12.1	8.6
Ammonia	17.4	6.0	21.6

Table 4. Proportions of various types of amino acids in taste-modifier and chemostimulatory proteins (calculated from data in Table 3).

Protein	Nonpolar (%)	Polar, uncharged (%)	Basic (%)	Acidic (%)	Ammonia (%)
Miraculin	47.2	18.0	14.4	20.4	17.3
Monellin	44.1	14.0	17.3	24.7	6.7
Thaumatin	45.7	26.6	11.7	16.0	13.0

these proteins raises an interesting question about whether peripheral adaptation occurs in these cases. Careful evaluation of the time course of persistence of the sweet taste of monellin and of thaumatin have not been made and should receive attention.

Summary

Three taste-active proteins have recently been discovered. It is proposed that two of these (monellin and thaumatin) should be classified as chemostimulatory proteins because of their sensory effect; these two proteins taste intensely sweet. The third protein (miraculin), a taste-modifier protein, changes the normal sour taste of acids to sweet.

The taste-modifier protein, miraculin, occurs in the fruit of the tropical plant *Synsepalum dulcificum*. Though itself not sweet, it is able to change the taste of acids from sour to sweet after the tongue has been treated with the protein. Miraculin is a basic glycoprotein with a molecular weight of 44,000.

Monellin, a chemostimulatory protein, is found in the fruit of a different tropical plant, *Dioscoreophyllum cumminsii*. It has been characterized as a basic protein with a molecular weight of 10,700 that contains no carbohydrate. Thaumatin, another chemostimulatory protein, occurs in the fruit of a third tropical plant, *Thaumatococcus daniellii*. Like monellin, it is a basic protein that contains no carbohydrate. Its molecular weight is around 21,000.

Certain gross similarities among the three proteins have been noted. Their basic ionic character and some features of the amino acid compositions are similar. Little is known of the structural features of the chemostimulatory proteins that are required for eliciting their intense sweetness; they are of the order of 10^5 times more effective than sucrose. The precise role of the tertiary structure in their biological activity is not known but appears to be an important area for further study. The relatively large size (11,000 to 21,000 molecular weight) of the chemostimulatory proteins provides indirect evidence that the initial interaction of these stimuli with taste receptor cells occurs at the plasma membrane.

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