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Production of Sweet Protein Transgenics with Monellin

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Abstract

Sweet proteins can replace artificial sweeteners, since they are natural sweeteners with low calories. Monellin, a naturally sweet Dioscoreophyllum cumminsii protein, produces sweet flavor, because carbohydrate has been proved to be important to its sweet taste. It is a heterodimeric protein that loses its activities during denature with its 94 amino acid residues with a molecular weight of 10.5 kDa. A promising sweetener that tastes sweet at pH 2–9 is proposed for Monellin, while high pH or heating after 70 ÚC or higher could denature this protein. Structural studies showed that the relation between these two subunits gives stability to this heterodimer monellin as opposed to the indígena. Monellin composed of one single polypeptide also has a flavor that is equivalent to the double-chain monellin. As flavor enhancer and high intensity sweetener, Monellin offers dual applications and was recommended for use in some countries. As the supply of naturally present monellin is small, its synthesis through transgenic organisms has been investigated thoroughly. Efforts were made in the different expression systems of bacterial, yeast and transgenic plants to produce this recombinant protein. Monellin development in transgenic fruit and vegetable products provides a viable approach to flavor and quality improvements. In prokaryotic (E. coli) and eukaryotic (tomato) systems, the stable and enhanced expression of synthetic sweet protein monellin has been reported. The temperature of the recombinant monellin protein remained strong and sweet over a range of temperature (up to 70℃) and intense pH levels.

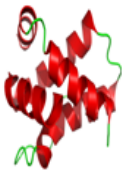
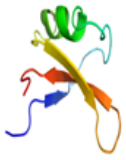
Key words: Artificial sweeteners, Heterodimeric protein, Monellin, Recombinant protein, Sweet proteins, Transgenic organisms

Introduction

Globally, the ingestion of high-calorie food, mainly comprised of various sugars and/or carbohydrates, affects millions of people, including type II diabetes, through obesity and related problems.Globally, the ingestion of high-calorie food, mainly comprised of various sugars and/or carbohydrates, affects millions of people, including type II diabetes, through obesity and related problems.As it is known that proteins do not cause insulin demand in those patients, while sucrose is (Table 1), so sweet protein has the abilities to substitute normal, healthy and low-calorie sweeteners for these artificial sweeteners[1].Together they were found to be an unusual protein inherent in sweet receptors, known from African berries. Such proteins were 100,000 times sweeter and several thousand times sweeter on a weight level than sugar on a molar basis.Neither carbohydrates nor altered amino acids are present[2].A natural sweet protein, Monellin as a human taste receptor derived from Dioscoreophyllum cummussii West African berries and is particularly helpful in the

treatment of clinically impaired individuals.The introduction of monellin to the food could in some way solve the problem. The food and beverage industries have therefore paid great attention to monellin.Protein chemists previously described monellin and deciphered its original AU4 structure.Nevertheless, due to its small environments and uncertainty in high temperatures and its accident pH, this low-calorie natural sweetener is restricted and causes sweet flavor loss.The relation between the two heterodimers will enhance monellin stability[3]. Structural tests have shown the same fashion and sweetness of monellin, consisting of one single polypeptide, as the double chain monellin, but stable as the indigenous equivalent.

Table 1: Structural details of important sweet-proteins known to be mankind

	Thaumatin	**Monellin**	**Mabinlin**	**Pentadin**	**Brazzein**	**Curculin**	**Miraculin**
Source	*Thaumatococcus denielli* Benth	*Dioscoreophyllum cumminsii* Diels	*Capparis masakai* Levl	*Pentadiplantra brazzeana* Baillon	*Pentdaplandra brazzeana* Baillon	*Curulingo latifolia*	*Richadella dulcifica*
PDB	3WXS	1MOL	2DS2	2BRZ	1BRZ	2DPF	3IIR
Structure							
Sweetness (weight basis)	3000	3000	100	500	2000	550	-
Molecular wt. (KDa)	22.2	10.7	12.4	12	6.5	24.9	98.4
Residue length	207	45 (A chain) 50 (B chain)	33 (A chain) 72 (B chain)	54	54	114	191
Active unit	Monomer	Dimer (A + B)	Dimer (A + B)	Monomer	Monomer	Dimer (A + A)	Tetramer (A + A + A + A)

Monellin, a sweet protein Dioscoreophyllum cummussii, elicits a sweet flavor, mainly because carbohydrates were recorded in 1969. In 1972, the Monell Chemical Senses Center in Philadelphia, USA, isolated it and was functionally characterized as a protein.It is 100 times as sweet as sugar on the molar basis.It is a heterodimeric protein with a molecular weight of 94 amino acid residues of 10.5 kDa that loses the activity in denaturation.Monellin is suggested to be a promising sweet-tasting sweetener with the pH of 2-9, while it can be denatured at or above a high pH or heat above 70℃. This means its application in the food industry is limited by its instability at high temperatures or pH limits.Monellin-protein single chain containing two polypeptides, i.e. chain A & B paired with a bonding of Gly-Phe dipeptides.The mutagenesis directed at the site was commonly used to alter and improvise the protein's functional characteristics[4].Monellin in vitro single chain is also shown to be a sweet wild type and more stable protein at high temperatures.The sweetest protein group

known to mankind is Monellin and its Single Chain Derivative (MNEI).While less is known about this monellin protein, extensive studies to improve protein sweetness and thermostability through gene editing and modification have been carried out[5].A researcher developed the new AU5 mutants of MNEI with increased sweetness in post-critical tests of surface electrostatic potentials.In addition, the E23Q stabilizing mutation was the most motivating solution, which resulted in a building with enhanced properties which gathered extreme sweetness to a high thermal stability, depending on the pH.The resulting mutant had only 0.28 mg/98L (25 nM) sweetness threshold and was the best known sweetener. Therefore, the new proteins were purified by X-ray crystallography and structures of the most solid mutants were determined.Some sweetness improvements in this protein have previously been recorded with some modifications such as G1 M, E2 M, and E2N. The sweetness and solubility of acid medium has improved considerably due to another mutation in the monellin protein like Y65R.The transition from MNEI into tobacco resulted in an increase in thermostability and stable shapes[6]. In order to ensure thermal stability, some MNEIs have successfully been converted into tobacco plant chloroplasts, following a circular dichroism study.Nonetheless, a detailed analysis of these mutants has not been performed on the thermal tolerance and denaturation, and sensory assessment[7].Both A and B of the polypeptides, covalently, have the following links, with a minimum of 44 and 50 amino residues: Chain A(44aa): GEWEIIDIDIGPFTQNLGKFAVDEENKIGQYG RLTFNKVIRPCMKKTIYEE.A secondary structural confirmation of 5 β sheets and a 17-residue alpha helix has been published, as clearly shown in resolute crystal structures of monellin.Under vapor diffusion of 20% ethanol these crystallographic structures have become a protein buffer solution.Two entire monellin protein molecules are found in the asymmetric carbon form.Monellin crystal protein pattern divide at least 2.5 Å, indicating the correlation of structural analyses with atomic resolution through X-ray crystallography.

1. Microbial Expression Systems:

Monellin is part of a family of extremely sweet tropical proteins. The D. cumminsii plant is a determined process; efforts have been made in various systems of expression to generate this recombinant protein. One of the most efficient systems of secretion expression in Bacillus subtilisis found among microbes and was systematized because of its numerous merits[8].The most important development is its ability to isolate and assimilate protein in comparatively pure form right into the culture medium.However, in biologically active systems, the established foreign proteins remain elusive. Protein secretions that envelop the production of inactive integration bodies during over expression of heterologous genes in Escherichia coli[9].However, in biologically active systems, the established foreign proteins remain elusive. Protein secretions that envelop the production of inactive integration bodies during overexpression of heterologous genes in Escherichia coli.As B. subtilis is not a pathogen in people; it represents a biologically healthy state. Several transgenes were expressed efficiently in B. subtilis recombinant monelline protein and enhanced yields were obtained.

2. Yeast Expression Systems:

For the synthesis of pharmaceutical and industrial proteins, yeast expression systems have been extensively used.However, in non-Saccharomyces yeasts, recombinant protein expressions and purification, like Pichia pastoris or Hansenula polymorphs and Kluyveromyces lactis lactose production have frequently exceeded[10].Single-chain monellin was synthesized using DNA synthesizers and cloned to the GAPDH promoter and terminator expression pGAP expression vector and converted to S. cerevisiae strains[11].As glucose was reduced and ethanol activated the ADH2 gene, monelline was successfully manifested in transformed yeast cells.On expression in ABl 10 a recombinant yeast strain using process machinery, the large scale of the purification of single-chain monellin was achieved. Monellin expression was measured by densitometry to be around 10% of the total protein.In addition, the purification yield of HPLC gel filtration and the purity were measured at 45% and 95% respectively. Finally, a sweet flavor was obtained from 54 g of distilled recombinant SCM.Because monelline was a highly essential protein, the basics were used as an efficient cleansing method. One of the researchers expressed the SCM for improved heat and sweetness of monellin, had two polypeptides connected to a glycine residue in Candida.Between the C gene promoter and terminator fragments cloned from C, the SCM gene was added. This promoter defective marker gene has enabled vectors to be integrated in either UrA3 or rDNA gene locus in high copy numbers.SCM was produced to a high level of over 50% of the total soluble protein with an expression level.The high-level expression of monellin protein and its cost-effective purification method allow its industrial production to be economically viable. In the last few years, P. pastoris developed SCM.

3. Transgenic plants as an Expression system:

A glycemic index of 0, which can be used as a natural product in the food industry and also be eaten by diabetics, is shown to be monellin protein.But because it is expensive to produce monellin from the original source, it is very exciting to produce sweet protein "monellin" in vitro through transgenic methods and new techniques in gene editing.Monellin production in transgenic fruits and vegetables is an alternative strategy for improving its flavor and quality. Previous attempts at monellin synthesis using recombinant technology were unsuccessful due to its low levels of plant expression.In transgenic salad and tomato plants Monellin was made[12]. A monellin single-chain gene encoding all polypeptide chains linked to a hinge sequence was propelled and transferred into lettuce and tomatoes by constitutive and fruit-rearing promoters.Monelline in tomatoes and transgenic lettuce was expressed and the leaves and fruits were respectively strongly monelline protein accumulating.Aiming at the expression of the monelline gene on the tomato fruits were the fruit-specific and whole plant expression.The latter was the promoter of CaMV, which operates in different plant organs.The protein has been derived from the pericarp of tomatoes or leaves and has been tested with ELISA.A 50 percent red and red-ripe transgenic tomato containing the promotor E8 and monellin, and a transgenic salad blade containing the promoter and monellin gene were used for re-combining monellin.The protein has been derived from the pericarp of

tomatoes or leaves and has been tested with ELISA.In order to improve monellin production at the tomato pericarp, transgenic tomatoes were exposed to ethylene. Monellin was measured at 23.9 μg / g fresh weight and was significantly higher in untreated transgenic fruits in ethylene-treating transgenic tomato.According to a study, it has been reported that In the prokaryotic (E. coli) and eukaryotic (tomato) systems, the synthetic sweet protein monellin had been stable and improved than previously noted.The thermostatic recombinant monellin protein maintained high sweetness over a range of temperatures (up to 70℃) and intense pH values.T2 lines profiled transcripts showed improved expression of monellin that had a positive correlation with the profiles of its protein expression that Western blots emphasized.The heterologous expression of monellin driven by the fruit-specific promoter, E8, has not resulted either in vegetative or fruit development parameters for transgenic tomato lines to produce phenotypic anomalies.In order to analyze the levels of mRNA expression of monellin in matured T2 lines of fruit, RT-PCR was performed.For WT tomatoes there was no similar amplification. Monellin transcript abundance was standardized to the inner regulation of tubulin. Two lines (T2-5; T2-14) monitored maximum levels of transcribed expression in the 24 exponential PCR cycle phase.Triplicate studies with different bio-logical replicates were performed.In order to detect the presence of monellin protein on transgenic and WT lines, ELISAs were used with polyclonal antimonellin.Independent triplicate experiments were performed and each sample was duplicated.Standard deviations have been found for statistical errors.Results from ELISA studies have been confirmed through an immuno-blot study by comparing signal to diluted monellin for each variant.The average protein concentration was 60 lg / mg (2,06) and 54 lg / mg (2.58), respectively, for the transgenic lines T2-5 and T2-14.Other transgenic lines also indicated monellin expressions, i.e. T2-4 (51 1.54), 243 T2-7 (47 1.78), T2-8 (45 1.96).Immuno-blotting of total fruit protein extract from the wild type and transgenic plants which express monellin (after the major protein Rubisco has been removed) was performed. Even untransformed wild-type fruit there was no detectable monellin protein which suggests that monellin transgene was not available.The calculated yield of monellin / mg protein equals 60 lg of soluble protein derived from the monellin-expressing transgenic tomato fruit.Fruit flavor and post ripening (10 days) assessments show a distinct or important sweetness compared with WT of T2-5 and T2-14 out of five transgenic lines. The sweetness of the tomato pericarp of fruits of the transgenic T2-5 and T2-14 lines was highly distinguishable from the WT fruit in all five panelists.The fruits from linesT2-5 and T2-14 were distinguished by a clear after taste that persisted in the mouth after swallowing.Transgenic tomatoes with heterologous expressions of monellin are sweeter than WT (Fig. 1)[13].

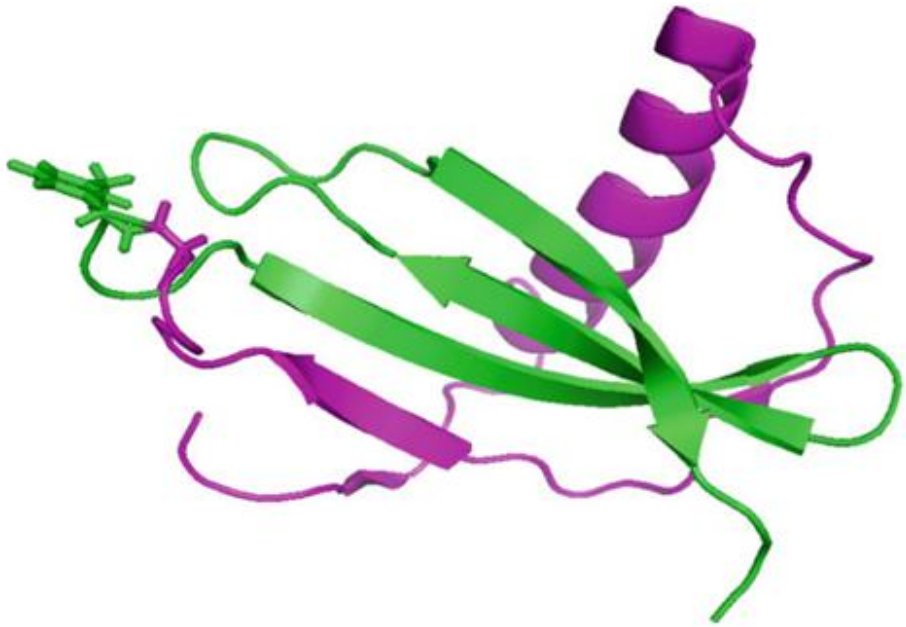


Fig.1: Diagrammatic representation of monellin 3D structure showing its two polypeptide chains.

Conclusion

High calorie food consumption causes obesity and related problems primarily through intake of refined sugars that ultimately result in high risk of diabetes of type II, heart disease, and other types of cancer, sleep apnea and arthritis.Sweetened beverages and other carbohydrate rich foods are the main sources of these excess carbohydrates.Although adding artificial sugar-free sweeteners may replace sweetness, there are still other side effects.The use of monellin as a natural sweetener would therefore become an ultimate alternative, as a low-calorie carbohydrate-free protein.Nevertheless, the purification of monellin protein is a tough task confined to ecosystems.In addition, both monellin subunits, i.e. chains A (45 amino acids that form three anti-parallel strands) and B (50 amino acids that make up two alpha-helix strands), are bound by uncovalent interactions which lead to their unstable existence.To minimize these problems, reassembling Native Monellin as a single-chain recombinant protein provides increased stability at high temperatures and intense pH and helps maintain its sweet flavor.Therefore, it is beneficial to assign correct monellin expressions for this important food supplement to a large-scale production.

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