Investigation of taste-altering mechanism: A model Study for sweet taste providing activity of miracle fruit

味覚改変作用機構の解明:ミラクリンフルーツの甘味提供機構をモデルとして

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Abstract

Miraculin (MIR) is a kind of taste-modifying glycoprotein, which can modify a sour taste into a sweet taste, isolated from a red fruit called Richadella dulcifica, also named as miracle fruits, a native shrub in tropical West Africa. It is a homodimer that consists of 2 glycosylated 191-amino acid polypeptides that are cross-linked by disulfide bonds. MIR has unique taste-modifying properties. Though flat in taste at neutral pH, it shows taste-modifying activity to convert sourness to sweetness at acidic pH. Although this interesting sensory effect has been previously characterized, the molecular mechanism underlying the taste-modifying action of MIR is unknown.

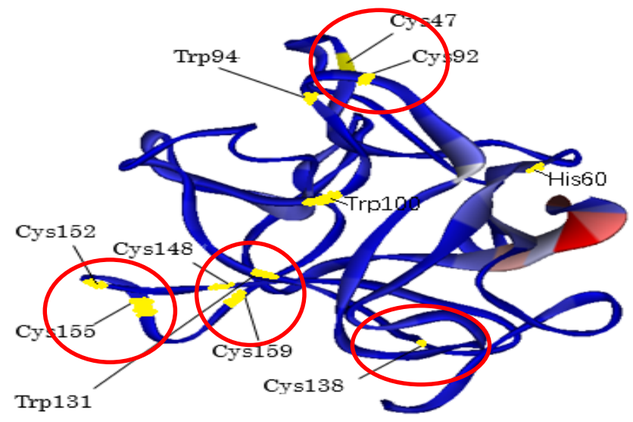
Photo of miracle fruits

Introduction

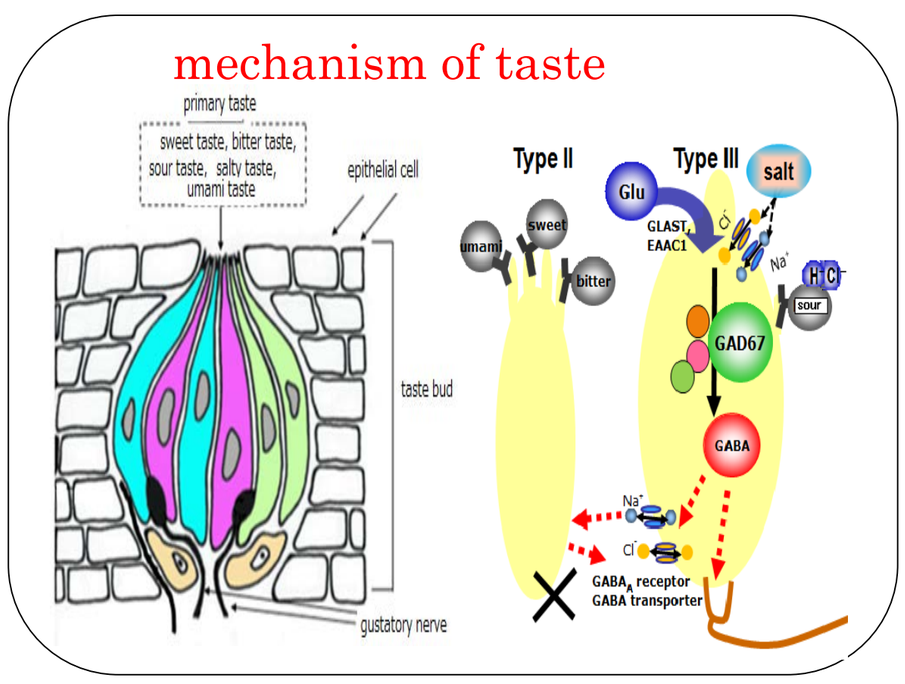
The position of S - S bonds:
①C47-C92, ② C152-C155,
③ C148-C159, ④ C138-C138(subunit)
The basic character of the MIR monomer:
191 amino acid residues,
Molecular weight is 24600Da (13.9% of sugars)
The dimer can modify a sour taste to a sweet taste, but the monomer can not.

S.Theerasilp, Y. Kurihara (1988
J. Biol. Chem. 263: 11536–9)

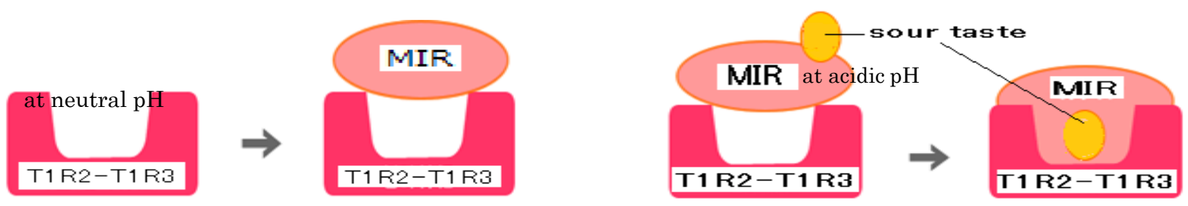
The amino acid sequence of miraculin

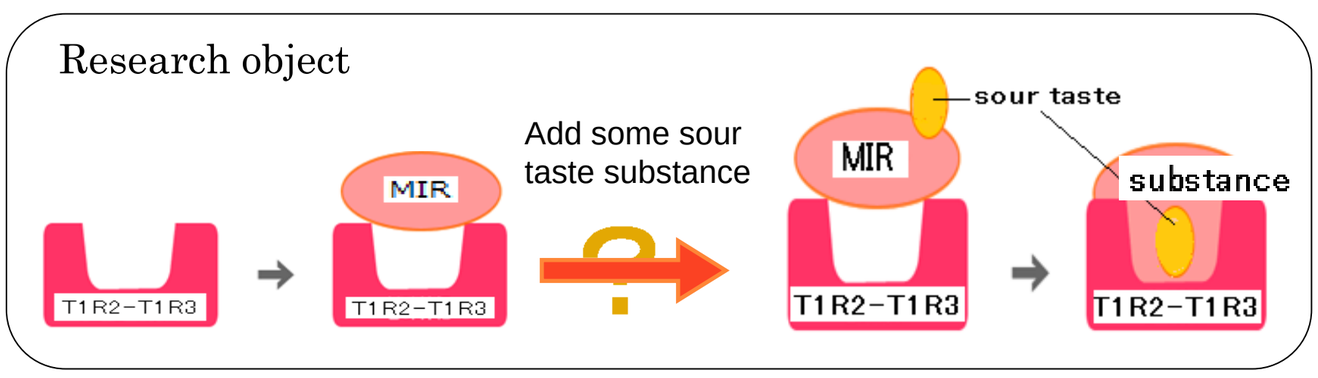


Simulation model of MIR monomer 3D structure by Discovery Studio® 2.5
(Template model: Kunitz (STI) type inhibitor, 1R8N)



The hypothetical mechanism of the MIR





Method and result

1. Sensory evaluation: We got the results by method, with sensory evaluation the crude extract from the miracle fruits.

Confirmation of the activity of miraculin

	Sour taste	Sweet taste
Ascorbic acid	-	++
Citric acid	+	+++
Acetic acid	-	+
Malic acid	-	+
Lactic acid	-	+

The PSE (Point of Subjective Equality) of the 4 kinds of the organic acid composition, when we can feel sweet taste.

	クエン酸		リンゴ酸		酢酸		アスコルビン酸	
甘味を呈する範囲	下限	上限	下限	上限	下限	上限	下限	上限
濃度 % (g/v)	0.01	0.05	0.008	0.038	0.007	0.037	0.021	0.103
mol濃度 (mmol/L)	0.52	2.60	0.60	2.83	1.17	6.16	1.19	5.85
pH	3.42	2.96	3.48	3.07	3.87	3.48	3.63	3.23
pKa	3.15 , 4.77 , 6.40		3.4, 5.1		4.76		4.17	

which sample can make you feel strongest sweet taste?

パネル	試料		
	0.01%クエン酸	0.008%リンゴ酸	0.007%酢酸
A	1	3	2
B	2	1	3
C	1	2	3
D	3	1	2
E	1	3	2
F	3	2	1
合計	11	12	13

which sample can make you feel stronger sweet taste between 2 different concentration compared with 0.8% sucrose?

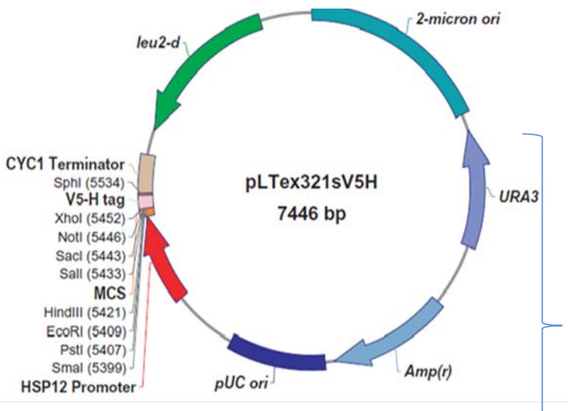
パネル	試料		
	0.01% クエン酸	0.05% クエン酸	0.8%スクロース
A	2	3	1
B	3	1	2
C	2	3	1
D	3	1	2
E	3	1	2
F	2	1	3
G	2	3	1
H	3	2	1
I	2	3	1
J	2	1	3
K	3	1	2
合計	27	20	19

パネル	試料		
	0.008% リンゴ酸	0.038% リンゴ酸	0.8%スクロース
A	2	3	1
B	3	1	2
C	2	3	1
D	3	1	2
E	1	3	2
F	2	3	1
G	2	1	3
H	2	3	1
I	2	1	3
J	2	3	1
合計	21	22	17

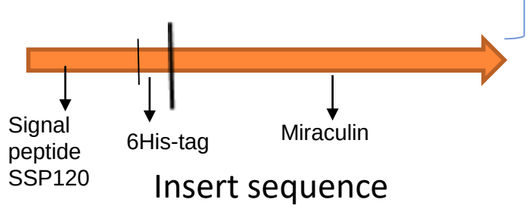
パネル	試料		
	0.007% 酢酸	0.037% 酢酸	0.8%スクロース
A	1	3	2
B	3	1	2
C	3	2	1
D	1	2	3
E	1	3	2
F	1	3	2
G	2	3	1
H	3	2	1
I	1	3	2
J	3	1	2
合計	19	23	18

パネル	試料		
	0.021 %アスコルビン酸	0.103% アスコルビン酸	0.8% スクロース
A	3	2	1
B	3	1	2
C	2	1	3
D	2	1	3
E	2	3	1
F	3	1	2
G	2	3	1
H	3	2	1
I	3	2	1
J	1	3	2
K	3	2	2
合計	27	21	18

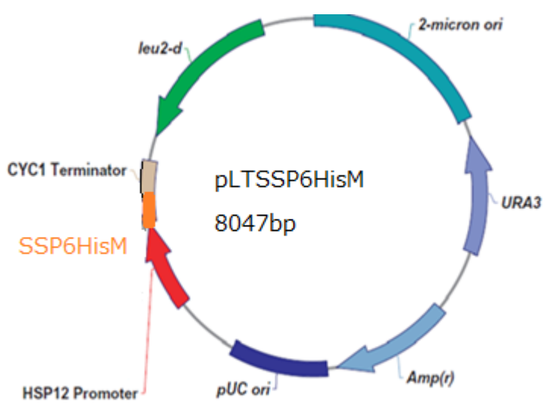
2. Plasmid construction



Expression vector map



Insert sequence



Expression plasmid map

Conclusion and discussion

No significant differences were noted in the results neither among the 4 kinds of organic acid composition nor between the two different kinds of concentration of the same organic acid.
But we can make sure about the unique taste-modifying activity of the MIR with 4 kinds of the organic acid composition.
We constructed the plasmid pLTSSP6HisM to express the recombination miraculin in yeast cells.
We`ll purify the MIR and re-MIR, and do the CD spectroscopic analysis to try to know the conformational change of miraculin acid-response. At last we`ll try to figure out the 3D protein structure of miraculin.