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Review

Molecular mechanisms of the action of miraculin, a taste-modifying protein

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ABSTRACT

Miraculin (MCL) is a homodimeric protein isolated from the fruits of *Richadella dulcifica*, a shrub native to West Africa. Although it is flat in taste at neutral pH, MCL has taste-modifying activity in which sour stimuli produce a sweet perception. Once MCL enters the mouth, strong sweetness can be detected for more than 1 h each time we taste a sour solution. While the human sweet taste receptor (hT1R2–hT1R3) has been identified, the molecular mechanisms underlying the taste-modifying activity of MCL remain unclear. Recently, experimental evidence has been published demonstrating the successful quantitative evaluation of the acid-induced sweetness of MCL using a cell-based assay system. The results strongly suggested that MCL binds hT1R2–hT1R3 as an antagonist at neutral pH and functionally changes into an agonist at acidic pH. Since sweet-tasting proteins may be used as low-calorie sweeteners because they contain almost no calories, it is expected that MCL will be used in the near future as a new low-calorie sweetener or to modify the taste of sour fruits.

Contents

1. Introduction

Sweetness, the most popular of the basic tastes, is representatively derived from carbohydrates, a source of energy commonly found in our daily foods, and the sweet sensation also acts as a nutritionally important signal. However, excessive intake of carbohydrates may cause lifestyle-related diseases. These current nutritional crises have led to the development of low-calorie sugar substitutes. Various artificial sweeteners have been developed to date and are widely used in food industrial products, such as soft drinks and confectioneries.

Typical examples of sweet substances include saccharides, such as sugar; amino acids, such as glycine and D-tryptophan; and artificial sweeteners, such as aspartame and saccharin. Most proteins, which are macromolecules, tend to be flat in taste, but some proteins, such as monellin and thaumatin, are known to be intensely sweet [1]. These sweet-tasting proteins may be used as low-calorie sweeteners because they are perceived by humans as intensely sweet, but have almost no calories [2].

2. Miraculin, a taste-modifying protein

Richadella dulcifica, a shrub native to West Africa and a member of the Sapotaceae family, yields red berries that are similar in size to olives (Fig. 1). When sour foods containing acetic or citric acid are consumed after this fruit, they taste remarkably sweet. Sour lemon, for example, tastes as good as a sweet orange after consumption of *R. dulcifica* fruit. Indeed, humans taste a strong sweetness every time they consume a sour solution for more than 1 h after this fruit is consumed [3]. Because of this unusual property, the berry is known as the "miracle fruit". African natives have been using this miracle fruit to sweeten sour palm wine or fermented bread, allowing them

Abbreviations: GPCR, G protein-coupled receptor; hT1R, human T1R; MCL, miraculin.

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Fig. 1. The "miracle fruit", *R. dulcifica.*

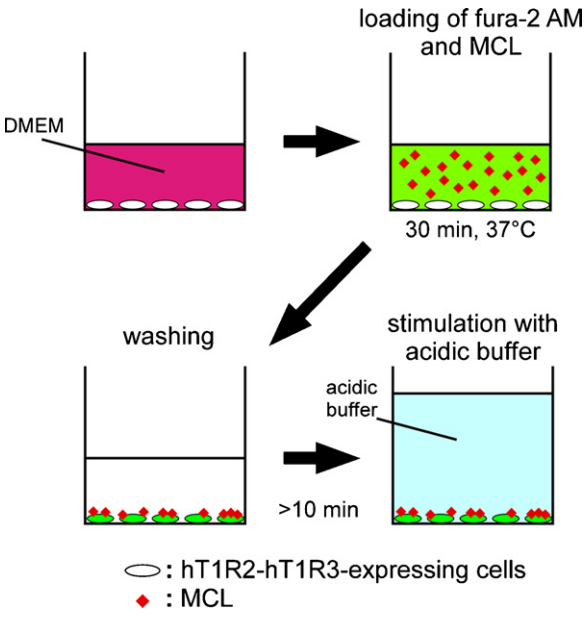

Fig. 2. Schematic illustration of the method used to mimic the conditions of human sensory tests using cultured cells. Illustration of a single well of a multiwell plate. MCL was pre-incubated with hT1R2–hT1R3-expressing cells together with a fluorescent calcium indicator (fura-2 AM) prior to acid stimulation. After washing with assay buffer, the cells were stimulated by the addition of the assay buffer adjusted to different pH values.

to easily consume these foods due to the sweetness produced by the miracle fruit.

The active component of *R. dulcifica* that modifies or converts sourness to sweetness, designated as miraculin (MCL), is a taste-modifying protein that exhibits extremely unusual properties and is famous for its unique taste characteristics [4]. MCL, which is purified from the fruit of *R. dulcifica*, is flat in taste. However, when held on the tongue, MCL causes subsequently consumed sour foods to taste sweet, resulting in the illusion that MCL "converts sour into sweet".

The complete amino acid sequence of purified MCL protein was characterized in 1989 [5], and the nucleotide sequence of the cDNA for MCL was revealed in 1995 [6]. MCL is a homodimeric protein that consists of polypeptides composed of 191 amino acid residues. The 2 subunits are held together by intramolecular disulfide bonds. The presence of 2N-linked oligosaccharides per subunit has also been confirmed [7].

3. Mechanism of action of MCL on the human sweet taste receptor

3.1. Activation of sweet taste receptors by pH reduction

Based on the phenomenon that a sweet sensation is evoked every time an acidic solution is consumed after MCL has been taken into the mouth, a functional scheme for the sweet inducing mechanism of MCL was initially proposed by Kurihara and Beidler [3]. According to this scheme, MCL is bound to membrane of taste cells near the sweet receptor site. The receptor membrane undergoes a structural change in the presence of protons (H^+), causing the sugar part of the MCL molecule to bind to the sweet receptor site in the membrane, thereby evoking a strong sensation of sweetness. In other words, the basis of the sweetness-inducing behavior under acidic conditions is the pH-dependent conformational changes of the receptor membrane that detect the sweet sensation [3].

However, at the time when the scheme was proposed, the molecular entity corresponding to the sweet taste receptor had not been identified, and therefore, experimental verification of this scheme was impossible. Moreover, while the human sweet taste receptor (hT1R2–hT1R3) has now been identified [8], there have been no reports describing the functional characterization of MCL, and the molecular mechanism underlying the taste-modifying activity of this protein has remained unclear for many years. Interestingly, the acid-induced sweetness of MCL was diminished in the presence of a sweet taste inhibitor, lactisole [9]. Lactisole is known to specifically inhibit the human sweet taste receptor (hT1R2–hT1R3) by interacting with the transmembrane domain of the hT1R3 subunit [10], suggesting that MCL acts in collaboration with the hT1R2–hT1R3 receptor. Based on these data, a cell physiological study using cultured cells expressing the human sweet taste receptor has been conducted in order to clarify the unusual properties of MCL and, more specifically, to uncover its curious acid-stimulated sweetness-inducing mechanism [11].

Based on the phenomenon that the taste-modifying activity of MCL can be detected for more than 1 h in human sensory tests [3], it was hypothesized that MCL directly and intensely binds to the human sweet taste receptor and activates the receptor as the surrounding pH decreases. To test this hypothesis, a reliable evaluation system was designed that mimics the conditions of sensory tests in a cell-based assay using cultured cells that express the human sweet taste receptor [11]. Specifically, MCL was pre-incubated with hT1R2–hT1R3-expressing cells together with a fluorescent calcium indicator (fura-2 AM) prior to acid stimulation. Subsequently, the cells were washed to remove both excess MCL and the indicator and then stimulated with acidic buffer to evaluate acid-induced sweet intensities (Fig. 2). We performed an assay under these conditions to evaluate the relationship between the pH value and the response of MCL-pre-incubated cells expressing the human sweet taste receptor (Fig. 3). When the MCL-pre-incubated cells were stimulated with acidic buffer (final pH: 5.0), the number of responding cells was significantly greater than that after the addition of neutral buffer (final pH: 7.0; Fig. 3A). As a control, it was also confirmed that the cell response after acid application was not detectable without either MCL pre-incubation (Fig. 3A, left) or the expression of hT1R2–hT1R3 (not shown). Moreover, the cellular response to the acidic buffer increased as the pH value of the acidic buffer decreased (Fig. 3B). Between pH 4.8 and 6.5, the response increased in a pH-dependent manner, whereas little response was observed at pH 6.5–7.4. At pH 5.7, the cells were half as responsive as they were at pH 4.8 (Fig. 3B). These results confirmed experimentally that MCL activates the human sweet taste receptor in a pH-dependent manner, i.e., more strongly as the acidity increases.

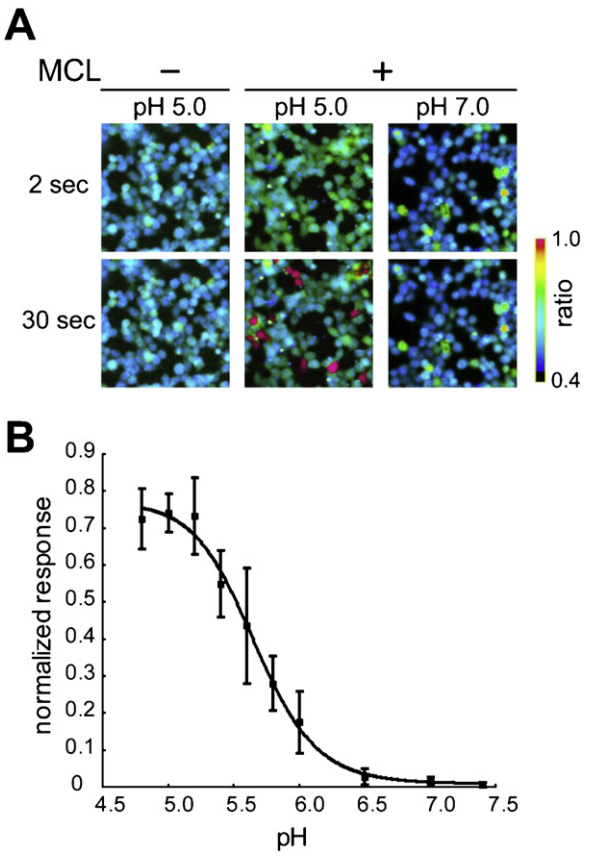

Fig. 3. MCL activates hT1R2–hT1R3 in a pH-dependent manner. (A) Representative ratiometric images of fura-2-loaded and MCL-pre-incubated hT1R2–hT1R3- and G15Gi3-expressing cells in response to acidic buffer (pH 5.0, center) and neutral buffer (pH 7.0, right). The pH values of the ligand solution were adjusted with citric acid such that the pH values after adding the ligands to the cells were as indicated. The top and bottom columns show the representative cell images obtained 2 and 30 s after acidic buffer application, respectively. The color scale indicates the F_{340}/F_{380} ratio as a pseudo-color. (B) Responses of MCL-pre-incubated hT1R2–hT1R3 expressing cells under different pH conditions. The number of responding cells was represented by the normalized response to 10 mM aspartame at pH 7.4.

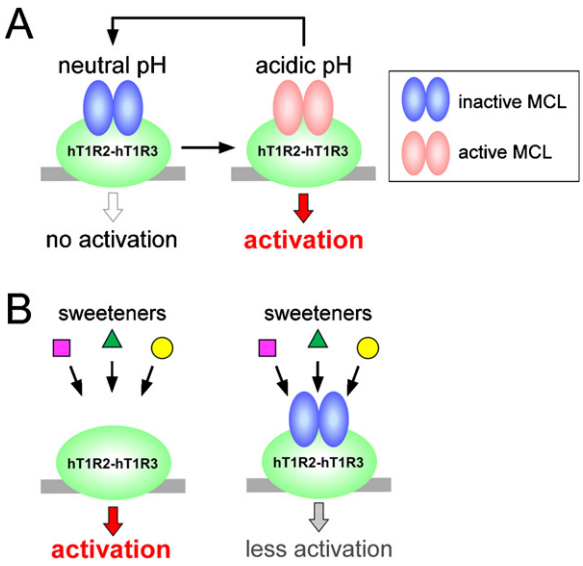

Fig. 4. Possible model for the taste-modifying activity of MCL. (A) MCL is bound to hT1R2–hT1R3 at neutral pH (left) and activates the receptor under acidic pH (right). When the pH reverts to neutral, MCL converts into its inactive form on the receptor (left). (B) A variety of sweeteners activate hT1R2–hT1R3 (left), but MCL inhibits this activation at neutral pH (right).

3.2. Effects of MCL on the human sweet taste receptor

As described above, an evaluation system using cultured cells functionally expressing the human sweet taste receptor uncovered the taste-modifying mechanism of MCL, i.e., the modification from sour to sweet taste (Fig. 4A). The results basically supported the sweetness-inducing mechanism of MCL suggested by the MCL activation model proposed over forty years ago [3], which reaffirms the insights of our visionary predecessors.

On the other hand, a number of new findings were also obtained from the assay using cells expressing the human sweet taste receptor [11]. Since MCL itself is flat in taste and evokes a sensation of sweetness only at acidic pH, it was naturally expected that MCL would have no effect on sweet taste receptor under neutral conditions. However, surprisingly, when another sweet substance was administered at neutral pH to MCL-pre-incubated cells expressing the human sweet taste receptor, the activity of the administered sweet substance was strongly inhibited in a dose-dependent manner for all sweet substances tested [11]. This strongly suggests that MCL binds directly to the region of the human sweet taste receptor molecule on the outside of the cell, even under conditions where a sweet sensation is not evoked (Fig. 4B) and that the interaction between MCL and the human sweet taste receptor is extremely strong. Thus, the question as to how MCL inhibits the human sweet taste receptor by binding to its extracellular domain may now garner new interest, and further studies are needed to clarify the unique aspects of MCL-mediated taste-modifying activity.

4. Conclusion

As described above, the development of an assay system using cells expressing the human sweet taste receptor permitted us to uncover a more complete picture of the unusual properties of MCL, i.e., modifying taste perception from sour to sweet. MCL is likely to be in equilibrium between being an agonist and an antagonist at acidic and neutral pH, respectively (Fig. 4). Therefore, based on many years of experimental results, this review has summarized the most probable molecular mechanisms for the activity of MCL as follows. Once humans taste MCL, it binds to hT1R2–hT1R3 and subsequently acts as an agonist every time a sour solution is tasted. MCL becomes an antagonist when the pH reverts to neutral and then suppresses the activation of the receptor by other sweeteners. MCL stays bound to the receptor and can reactivate it, consistent with the finding that the taste-modifying activity of MCL can last over 1 h. At present, experimental evidence has not elucidated whether this mechanism of action is caused by pH-dependent structural changes in MCL. However, the finding that the action of MCL on the human sweet taste receptor varies significantly depending on pH suggests the possibility of the dynamic structural changes in MCL molecules.

The sensation of sweetness evoked by MCL is refined and very pleasant. In addition, the development of a safe, novel calorie-free substance that tastes sweet would be ideal for those who suffer from lifestyle-related diseases. Thus, taste-modifying proteins have also been attracting attention in the food industry. The successful production of active MCL using *Aspergillus oryzae* [9], lettuce [12,13], or tomatoes [14–20] as a host has recently been reported. Therefore, MCL may have promise in the near future as a new low-calorie sweetener or to modify the taste of sour fruits.

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