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Short communication

Five amino acid residues in cysteine-rich domain of human T1R3 were involved in the response for sweet-tasting protein, thaumatin

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ABSTRACT

Thaumatin, a sweet-tasting plant protein, elicits a sweet taste sensation at 50 nM in humans but not rodents. Although it was shown that the cysteine-rich domain (CRD) of human T1R3 (hT1R3) is important for the response to thaumatin, the amino acid residues within CRD critical for response are still unknown. A comparison of the amino acid sequence (69 amino acid residues) of CRD between hT1R3 and mouse T1R3 (mT1R3) revealed sixteen amino acids that differ.

In the present study, we converted each of these sixteen amino acids in hT1R3 to their mouse counterpart and examined the response to thaumatin and sucralose using a cell-based assay. No significant decrease in the response to sucralose was seen among any of the sixteen mutants. However, five mutants (Q504K, A537T, R556P, S559P, and R560K) exhibited a significantly diminished response to thaumatin. The five critical residues involved in the response to thaumatin were dispersed in the CRD of hT1R3 and widely distributed when compared to brazzein.

The unique intense sweet-taste of thaumatin might be attributed to the different receptor activation mechanism compared to the small molecule sweetener sucralose.

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1. Introduction

Thaumatin is the one of the sweetest proteins known and used as a low-calorie sugar substitute as well as for medical purposes for lifestyle-related diseases. Thaumatin is 100,000-fold sweeter than sucrose on a molar basis and this intense sweetness makes thaumatin useful for unveiling the interaction between sweeteners and sweet receptors. As sweet-tasting proteins are too large to fit the cavity of the interaction sites for small sweeteners, the activation of sweet receptors by sweet-tasting proteins seems to occur in a different manner compared to other small sweeteners [1–3]. Previous mutational studies of thaumatin suggested that K67 and R82 are important to the sweetness of thaumatin, and mutations at R82 had a more deteriorative effect on sweetness than mutations at K67 [4].

The heterodimers comprising the subunits T1R2 and T1R3, which belong to a family of class C G-protein-coupled receptors, are known to function as sweet receptors [5–8]. Each subunit of sweet receptor possesses a large N-terminal domain (NTD) and a cysteine-rich domain (CRD), and followed by a seven-helix transmembrane domain (TMD). The CRD links the NTD and TMD. Previous studies have shown that sweet-tasting proteins as well as aspartame can be perceived by humans, apes, and Old World monkeys but not New World monkeys and rodents [9,10]. Species differences in the response to sweeteners would provide valuable information on the molecular mechanism by which sweet receptors function as well as aid the identification of interaction sites in receptors [10–14]. Recently, we have shown that the CRD within hT1R3 is important for the response toward thaumatin [14]. However, it remains unclear whether two sweet-tasting proteins, thaumatin and brazzein, interact with the same amino acid residues in the CRD of human sweet receptors.

In the present study, to clarify the amino acid residues within the CRD of hT1R3 critical for thaumatin reception and to clarify the mechanisms by which thaumatin activates sweet receptors, we performed site-directed mutagenesis in the CRD of hT1R3 relative to the CRD of mT1R3. The findings should help clarify the activation mechanisms of proteinous sweeteners and might lead to the design of new sweeteners.

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2. Materials and methods

2.1. Materials

Thaumatin I was purified from crude thaumatin powder as described previously [15]. Sucralose were obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan).

2.2. Site-directed mutagenesis of the CRD of hT1R3

The plasmid pcDNA3.3-hT1R3 was used as the template for mutagenesis [14]. Site-directed mutagenesis was performed using a KOD Plus DNA polymerase (Toyobo Co. Ltd., Osaka, Japan) with two synthetic complementary to opposite strands of oligonucleotide primers containing the desired mutation (Operon Biotechnologies, Tokyo, Japan, Supplement Table 1). The desired mutations were confirmed by DNA sequencing.

2.3. Functional expression of human sweet receptors

Human T1R2- and T1R3- or T1R3 mutant-containing plasmids were transiently transfected into HEK293 cells stably expressing the chimeric G-protein, $G\alpha_{16gust44}$ as described previously [14]. After the transfection, cells were seeded onto polylysine-coated 96-well culture plates (1.5×10^5 cells/well) (BD Biosciences, Bedford, MA) and incubated for 24 h. They were then loaded with 50 μL of 3 μM fluo-8 AM (ABD Bioquest Inc., Sunnyvale, CA) in Hank's balanced salt solution (HBSS) containing 20 mM Hepes and 1.25 mM probenecid for 30 min at 37 °C. The cells were incubated with 180 μL of 20 mM Hepes-HBSS containing 0.625 mM probenecid for 10 min at 37 °C. Stimulation was performed by adding 20 μL of agonist solution dissolved in 20 mM Hepes-HBSS. The response to sucralose (1 mM) or thaumatin (50 μM) was detected by measuring fluorescence (excitation at 495 nm and emission at 514 nm) using an Infinite F200 (Tecan Group Ltd., Männedorf, Switzerland) as described previously [14]. The response of

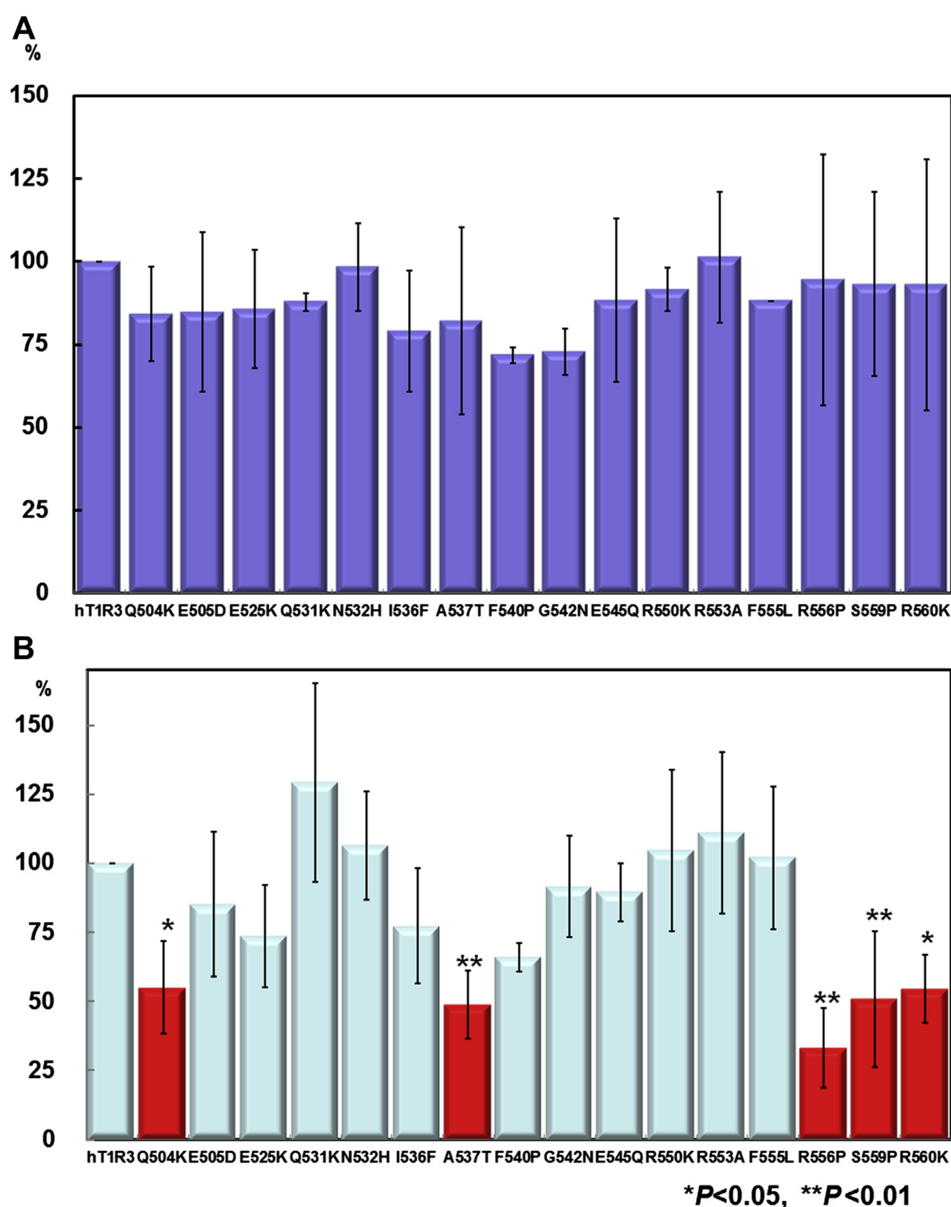

Fig. 1. Five amino acid residues in the CRD of hT1R3 affect the response to thaumatin. Human T1R2- and T1R3 mutant containing plasmids were transiently transfected into $G\alpha_{16gust44}$-expressing HEK cells and responses to sucralose (A) and thaumatin (B) were investigated by cell-based assay. The responses of 16 mutants were averaged and analyzed with a one-way ANOVA (analysis of variance). $^{*}P < 0.05$, $^{**}P < 0.01$.

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