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Distinct Contributions of T1R2 and T1R3 Taste Receptor Subunits to the Detection of Sweet Stimuli

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Summary

Animals utilize hundreds of distinct G protein-coupled receptor (GPCR)-type chemosensory receptors to detect a diverse array of chemical signals in their environment, including odors, pheromones, and tastants [1]. However, the molecular mechanisms by which these receptors selectively interact with their cognate ligands remain poorly understood. There is growing evidence that many chemosensory receptors exist in multimeric complexes [2–4], though little is known about the relative contributions of individual subunits to receptor functions. Here, we report that each of the two subunits in the heteromeric T1R2:T1R3 sweet taste receptor [2, 5–10] binds sweet stimuli though with distinct affinities and conformational changes. Furthermore, ligand affinities for T1R3 are drastically reduced by the introduction of a single amino acid change associated with decreased sweet taste sensitivity in behaving mice [11]. Thus, individual T1R subunits increase the receptive range of the sweet taste receptor, offering a functional mechanism for phenotypic variations in sweet taste.

Results and Discussion

It has been problematic to decouple the mechanisms of ligand binding from other processes that impact chemosensory receptor specificity, activation, and signaling, such as phosphorylation or G protein coupling. Recently, a number of taste receptors have been successfully deorphaned, offering the possibility of more fully characterizing receptor-ligand interactions within this group of GPCRs. For example, heteromeric T1R2:T1R3 taste receptors respond to sweet-tasting compounds such as sugars, high-potency sweeteners, and some D amino acids [2, 12–15], whereas T1R1:T1R3 heteromers comprise a umami taste receptor sensitive to L amino acids [12, 16]. Domains of human T1R2 and T1R3 are sufficient to confer sensitivity to some noncaloric sweeteners and sweet-tasting proteins to which rodents are indifferent [17, 18], but it remains unknown which of these receptor subunits participates in the binding of most sweet stimuli, including sugars. To specifically address this question, we have devised and executed a strategy for the direct analysis of T1R-ligand interactions.

Based on their homology to other class C GPCRs, such as metabotropic glutamate receptors (mGluRs) and γ-aminobutyric acid type B receptors ($GABA_BRs$) [19], we hypothesized that a binding site for sugars is localized within the long N-terminal domains (NTDs) of T1R2 and T1R3 (Figures 1A and 1D). We expressed and purified mouse $T1R2NTD_{B6}$ and $T1R3NTD_{B6}$ proteins, both of which were identical to the C57BL/6J (B6) alleles. Purified proteins either contained a C-terminal FLAG-tag ($T1R3NTD_{B6}$; Figures 1B and 1C) or were fused to maltose binding protein (MBP) at their N termini to increase the solubility of the NTD domain [MBP-$T1R3NTD_{B6}$, not shown; MBP-$T1R2NTD_{B6}$, Figures 1E and 1F]. A $T1R3NTD_{B6}$ variant with a single amino acid change, I60T, was similarly expressed and purified (Figures 1B and 1C).

T1R2 and T1R3 Taste Receptors Each Bind Sweet Ligands

We measured the interaction of two sugars and a noncaloric sweetener with $T1R3NTD_{B6}$, MBP-$T1R3NTD_{B6}$ and MBP-$T1R2NTD_{B6}$. To do this, we determined the concentration-response relationships for the peak intrinsic tryptophan fluorescence of the T1R NTD proteins by fluorescence spectroscopy upon titration of these sweet ligands. This technique is highly sensitive to changes in the local environment of fluorescent amino acid residues within a protein and is a good indicator of ligand binding and/or ligand-dependent conformational changes (e.g., [20, 21]). Glucose, sucrose, and sucralose decreased the peak fluorescence intensity of $T1R3NTD_{B6}$, exhibiting K_d values of 7.3 ± 0.7, 2.9 ± 0.4, and 0.9 ± 0.1 mM, respectively (Figures 2A and 2B, and Table 1). The interactions of these ligands with T1R NTDs is specific: cyclamate, which is perceived as sweet by humans but is not preferred by mice [22] and which appears to bind within the transmembrane domain of human T1R3 [17, 23], had no effect on the intrinsic fluorescence of mouse $T1R3NTD_{B6}$ (Figure 2B); and neither sucrose nor sucralose quench the intrinsic tryptophan fluorescence of another saccharide binding protein, MBP (though maltose does [20]; see Figure S1 in the Supplemental Data available with this article online). MBP-$T1R2NTD_{B6}$ also displayed a dose-dependent quenching of intrinsic fluorescence by glucose, sucrose, and sucralose, but not by cyclamate or the umami ligand L-glutamate (Figures 2C and 2D). However, the K_d values for MBP-$T1R2NTD_{B6}$ were different from those for $T1R3NTD_{B6}$ (Table 1). The presence of the MBP fusion appeared to have no effect on ligand binding, as the K_d values of glucose and sucrose

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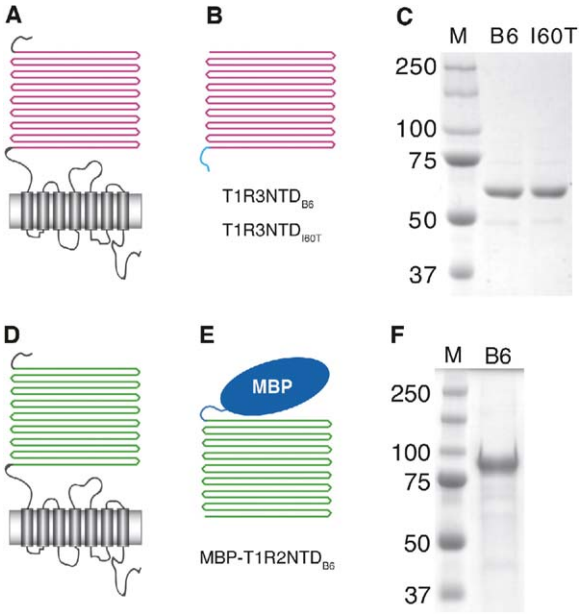

Figure 1. Expression and Purification of T1R NTD Proteins

(A) The NTDs of T1R3 (magenta) and (D) T1R2 (green), minus a short putative signal peptide and a cysteine-rich linker region (black), were expressed independently of the seven-transmembrane domain. Both cDNAs represent the C57BL/6J (B6) alleles.

(B) Purified T1R3NTD (B6 or I60T) was fused with a C-terminal FLAG-tag (cyan).

(C) Purified T1R3NTD$_{B6}$ or T1R3NTD$_{I60T}$, separated by SDS-PAGE and stained with Coomassie blue; M, molecular weight markers (in kDa).

(E) T1R2NTD$_{B6}$ (green) and T1R3NTD$_{B6}$ (not shown) were fused with maltose binding protein (MBP, blue) at their N termini.

(F) Purified MBP-T1R2NTD$_{B6}$, separated by SDS-PAGE and stained with Coomassie blue; M, molecular weight markers (in kDa).

interactions with MBP-T1R3NTD$_{B6}$ were nearly identical to those determined for T1R3NTD$_{B6}$ (Table 1); however, we cannot rule out the remote possibility that MBP differentially influences the two T1Rs. Our results demonstrate that the NTDs of both T1R2 and T1R3 bind sugars and sucralose at physiologically relevant concentrations.

Ligand binding stabilizes a conformational change in the NTDs of other class C GPCRs [19, 24]. We used synchrotron radiation circular dichroism (SRCD) spectroscopy, which is sensitive to the secondary and tertiary structure of chiral molecules such as proteins [25–27], to determine if T1R NTDs undergo a ligand binding-dependent change in conformation. The addition of 5 mM glucose, sucrose, or sucralose, but not 5 mM cyclamate, resulted in large changes in the SRCD spectrum of T1R3NTD$_{B6}$ (Figure 2E) and MBP-T1R3NTD$_{B6}$ (see Figure S2). The SRCD spectrum of MBP-T1R2NTD$_{B6}$ was also changed in the presence of glucose and sucrose, but not cyclamate (Figure 2F; sucralose was not tested); however, the changes are distinct from those seen for T1R3NTD$_{B6}$. Glucose did not change the conventional CD spectrum of MBP itself (see Figure S2), indicating that the changes in spectra were specific to the T1R NTDs. These results demonstrate that the NTDs of both T1R3 and T1R2 undergo a conformational change upon ligand binding, further supporting the distinct role of each subunit in the detection of sweet ligands. Higher resolution structural analysis will be needed to determine if these structural changes resemble those seen in the NTD of other class C GPCRs upon ligand binding [24].

A Single Nucleotide Polymorphism Associated with Reduced Sweet Taste Sensitivity Decreases Ligand Binding to T1R3

We sought to determine the physiological role of T1R3NTD in the detection of sweet stimuli. The *Tas1r3* gene has been suggested to be equivalent to *Sac* [2, 6–10], a single locus on mouse chromosome 4 [9, 28] that accounts for up to 80% of variation in sweet taste preference amongst inbred mouse strains [11]. Only one polymorphism significantly associated with saccharin sensitivity, T179C, results in a change in protein sequence, I60T [11]. The I60T change has been hypothesized to perturb receptor function by affecting ligand binding or interfering with the dimerization of T1R2 and T1R3 [6, 11]. However, the functional basis of the *Sac* phenotype has not been determined.

We examined the effect of the I60T change on the ability of T1R3NTD$_{B6}$ to bind sweet ligands. Glucose, sucrose, and sucralose, each at 5 mM, failed to change the SRCD spectrum of T1R3NTD$_{I60T}$ (Figure 3A), suggesting that this mutation interferes with ligand binding-dependent changes in receptor structure. However, glucose, sucrose, and sucralose each bound T1R3NTD$_{I60T}$ (Figures 3B and 3C) with the same relative order of K_d values as seen for T1R3NTD$_{B6}$. Strikingly, the T1R3NTD$_{I60T}$ dose-response curve for each ligand was shifted to the right compared to the equivalent for T1R3NTD$_{B6}$ (Figures 3D–3F), resulting in a dramatic increase in K_d (Table 1). Therefore, the I60T change alters the function of the sweet taste receptor by perturbing the interactions of T1R3NTD with sweet ligands and may further alter the subunit's ability to undergo a conformational change upon ligand binding. These data provide functional validation that polymorphisms in the *Tas1r3* gene are synonymous with *Sac*. Furthermore,

Table 1. Sweet Ligand Interactions with T1R NTDs

Protein	Sucralose (mM)	Sucrose (mM)	Glucose (mM)
MBP-T1R2NTD$_{B6}$	0.052 ± 0.004	15 ± 5	2.6 ± 0.2
T1R3NTD$_{B6}$	0.91 ± 0.15	2.9 ± 0.4	7.3 ± 0.7
MBP-T1R3NTD$_{B6}$	ND	3.4 ± 0.4	8.2 ± 1.5
T1R3NTD$_{I60T}$	6.9 ± 0.9	20 ± 3	32 ± 5

K_d values (mM; mean ± SEM). ND, not determined.

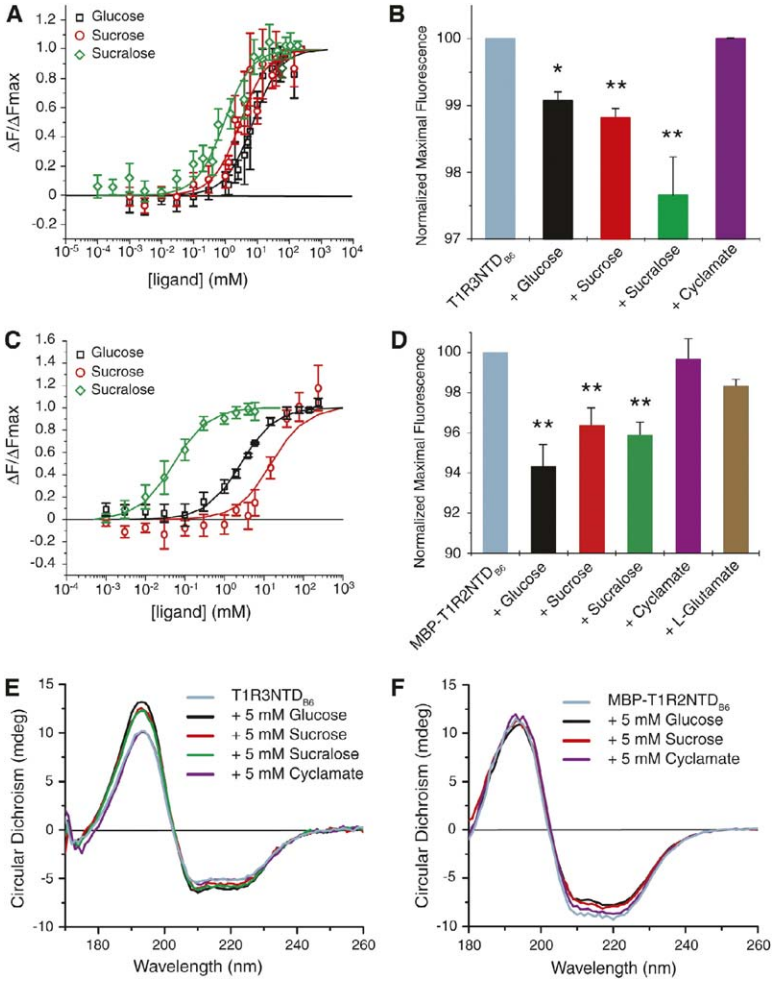

Figure 2. The NTDs of Mouse T1R3 and T1R2 Bind Sweet Stimuli

(A) The intrinsic tryptophan fluorescence intensity of T1R3NTD$_{B6}$ was measured as a function of ligand concentration for glucose (black), sucrose (red), and sucralose (green).

(B) Normalized maximal fluorescence intensity of T1R3NTD$_{B6}$ before and after titration of ligand, including cyclamate (plum). Data were compared by one-way ANOVA ($F[4,23] = 13.7$ [$p < 0.0001$] with a post-hoc Dunnett Multiple Comparison (*$p < 0.05$, **$p < 0.01$).

(C) The intrinsic tryptophan fluorescence of MBP-T1R2NTD$_{B6}$ was measured as a function of ligand concentration for glucose (black), sucrose (red), and sucralose (green).

(D) Normalized maximal fluorescence intensity of MBP-T1R2NTD$_{B6}$ before and after titration of ligand, including cyclamate (plum) and the umami ligand L-glutamate (brown). One-way ANOVA [$F(5,23) = 11.61$, $p < 0.00001$]) with a post-hoc Dunnett Multiple Comparison (**$p < 0.01$).

(E) SRCD spectra of T1R3NTD$_{B6}$ alone (gray), with glucose (black), sucrose (red), sucralose (green), or cyclamate (plum). All except cyclamate caused a large shift in the SRCD spectra around 195 nm (positive shift) and 205–230 nm (negative shift).

(F) SRCD spectra of MBP-T1R2NTD$_{B6}$ alone (gray), with glucose (black), sucrose (red), or cyclamate (plum). Interestingly, the ligand binding-induced spectral changes are distinct for the two subunits: T1R3NTD$_{B6}$ exhibits a positive shift in the SRCD spectrum around 195 nm and a negative shift between 205 and 230 nm upon ligand binding, whereas MBP-T1R2NTD$_{B6}$ showed no change around 195 nm and a positive shift between 205 and 230 nm.

Error bars indicate SEM.

they offer a mechanistic explanation, a decrease in the ability of T1R3 to bind ligand, for the contribution of this I60T allele to the phenotypic variation in sweet taste preference observed in mice. Interestingly, the intrinsic fluorescence of T1R3NTD$_{I60T}$ was quenched by 5 mM ligand, albeit to a reduced degree, in the absence of a concomitant conformational change. From this observation we conclude that ligand binding to the T1R NTD is distinct from a binding-dependent conformational change or subunit multimerization, thus offering a unique opportunity to dissect these interdependent stages of receptor activation.

Conclusions

These studies offer fundamental new insights into the receptor mechanisms used to detect and transduce those taste stimuli, such as sugars, that are preferred by most mammals. By decoupling the contribution of ligand binding and its associated conformational changes within the NTDs from subsequent events in the activation of T1R2:T1R3 receptors, we show that both subunits are capable of binding sugars and sucralose. This observation raises important questions regarding the mechanism by which T1Rs transduce different stimuli. For example, does the efficient activation of downstream effectors require the occupation of binding sites on both T1R2 and T1R3? Or are distinct subsets of sweet stimuli more efficiently transduced through binding to one subunit or the other? Evidence from human-rodent chimeric T1Rs would suggest that at least for some sweeteners that are preferred only by higher primates, the two subunits do play distinct roles in the detection and/or the activation of T1R2:T1R3 [17, 23]. In these studies, the NTD of human T1R2 was required for activation of chimeric receptors by neotame or aspartame, whereas the transmembrane domain of human T1R3 conferred sensitivity to cyclamate. In our studies, T1R3NTD$_{B6}$ binds sucrose with higher affinity than does T1R2NTD$_{B6}$, though the relationship is reversed for glucose. This is an intriguing observation, as humans and mice find sucrose sweeter than glucose on a molar basis (e.g., [2, 12, 29, 30]). It is possible that sugar interactions with T1R3 are more efficacious than they are with T1R2, thereby tightly linking the relative ability of sugars to bind T1R3 with their ability to activate the heteromeric receptor. In this context, it is particularly intriguing that the I60T change in T1R3 reduces this subunit's affinity for sugars (Figure 3) and is also significantly correlated with reduced sweet taste sensitivity in behaving mice [11].

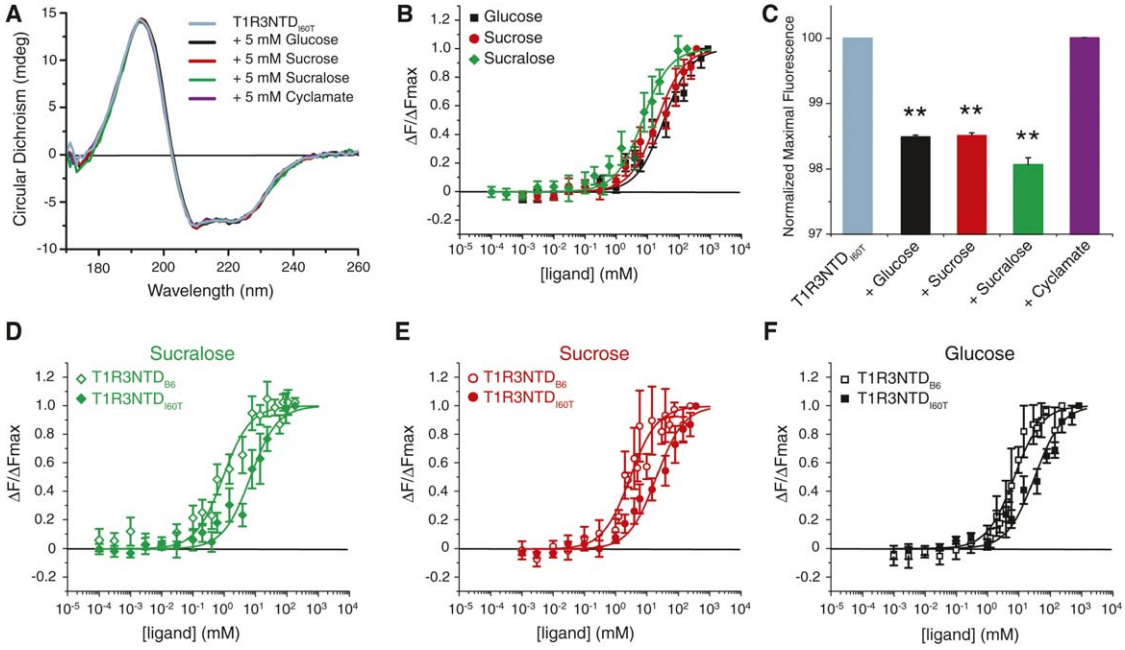

Figure 3. T1R3NTD$_{I60T}$ Has a Reduced Affinity for Sweet Ligands

(A) SRCD spectra of T1R3NTD$_{I60T}$ alone (gray), with glucose (black), sucrose (red), sucralose (green), or cyclamate (plum). None of the four stimuli caused a shift in the SRCD spectrum.

(B) The intrinsic tryptophan fluorescence intensity of T1R3NTD$_{I60T}$ as a function of ligand concentration for glucose (black), sucrose (red), and sucralose (green).

(C) Normalized maximal fluorescence intensity of T1R3NTD$_{I60T}$ before and after titration of ligand. One-way ANOVA [$F(4,19) = 46.0$ ($p < 0.0001$)] with a post-hoc Dunnett Multiple Comparison (**$p < 0.01$).

(D–F) Comparison of the dose-response relationships for T1R3NTD$_{B6}$ (open symbols) and T1R3NTD$_{I60T}$ (filled symbols) for sucralose (green), sucrose (red) and glucose (black).

Error bars indicate SEM.

However, it is clear from in vitro and in vivo studies that both T1R2 and T1R3 are required to create a fully functional saccharide receptor [2, 12–14]. Dimerization is also essential for other class C GPCRs. For example, the GABA$_B$R1 subunit alone contributes a high-affinity ligand binding pocket to heterodimeric GABA$_B$Rs [19], but the GABA$_B$R2 subunit both modifies the ligand sensitivity of its GABA$_B$R1 partner [31] and couples the activated receptor to intracellular G proteins (e.g., [32]). In contrast, each subunit in the homodimeric mGluR is competent to bind ligands with equivalent affinity [19], though they do exhibit a negative intersubunit cooperativity [21]. Our data also suggest that heteromerization is essential to create fully functional sweet receptors. The K_d values we report are slightly lower than would be predicted based on behavioral studies in rodents [14, 22, 33] or receptor activation assays [2] in which the EC$_{50}$s for sucrose range from ~50–200 mM (behavioral thresholds for sucrose are less than 25 mM [30]). Therefore, it is likely that processes other than ligand binding and its associated conformational change, such as heteromerization of the subunits, intersubunit conformational coupling, and coupling of the receptor to intracellular G proteins, impact the efficacy of T1R2:T1R3 activation. Homomeric T1R3 receptors have been suggested to function as low-efficacy sugar receptors in a small subset of taste cells [14]. Our study did not directly address whether T1R3 forms functional homomeric receptors in vivo. However, our data do indicate that any reduction in receptor efficacy may depend more on suboptimal receptor signaling, perhaps due to inefficient G protein coupling [17] or from the absence of obligate complementary conformational changes contributed by T1R2, than from an inability of T1R3 to bind ligands per se. The absence of some necessary intersubunit coupling may also explain why sugars do not activate or potentiate the T1R1:T1R3 umami receptor (though cyclamate does) [17]. A fuller understanding of the structural basis of ligand sensitivity and discrimination by chemosensory receptors, especially in the context of behaviorally-relevant genetic variation, will further illuminate the contributions of heteromeric receptors to strategies of stimulus coding in taste and other G protein-coupled transduction systems.

Supplemental Data

Supplemental Data include supplemental experimental procedures and two supplemental figures and are available with this article online at http://www.current-biology.com/cgi/content/full/15/21/1948/DC1/.

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