# Sweet/umami taste receptor-mediated chloride-sensation revealed by X-ray crystallographic analysis

Vertebrates sense organic nutrients in foods, such as sugars, amino acids, and nucleotides, by the sweet and umami receptors in the oral cavity. Taste sensation through these receptors promotes the intake of foods containing nutrients necessary for sustaining life. Sweet and umami receptors consist of taste receptor type 1 (T1r) proteins; the T1r2/T1r3 heterodimer serves as the sweet receptor, while T1r1/T1r3 serves as the umami receptor [1]. These receptors possess large extracellular domains that are responsible for the recognition of major taste substances, such as sugars and amino acids.

The mechanism by which these receptors recognize taste substances has long been unknown due to a lack of structural information. The most critical bottleneck in the structural analysis of these receptors is the preparation of protein samples. We previously found that the extracellular ligand-binding domain (LBD) of T1r2a/T1r3 from medaka fish was amenable to sample preparation by screening T1rs from various species [2]. The crystallographic structure of T1r2a/T1r3LBD was determined using SPring-8 **BL41XU** (Fig. 1(a)) [3]. The structure revealed the architecture of the ligand-binding sites in the receptor and the mechanism by which the receptor accommodated a wide array of amino acids at the binding sites. We also analyzed the structural difference between the amino acid-bound and ligand-free T1r2a/T1r3LBD by solution scattering using SPring-8 **BL45XU** and other equipment and found that the LBD undergoes a conformational change upon amino acid binding, likely inducing receptor responses resulting in taste sensation (Fig. 2) [2]. The crystal structure of medaka T1r2a/T1r3 is the sole structural information for T1rs reported to date and serves as a model for understanding T1r functions.

During the structural investigation of medaka T1r2a/T1r3LBD, we found the binding of an unknown substance in the vicinity of, but allosteric to, the amino acid binding site in the T1r3 subunit. The substance was most likely a chloride ion based on the coordination structure and distances (Fig. 1(a)). The effects of chloride on T1rs have never been investigated, although several examples have been reported for other receptors in the same protein family: class C G protein-coupled receptors (GPRCs). Therefore, we attempted to identify the chemical entity of the unknown substance, presumed to be a chloride ion, and examine its action on taste receptors [4].

First, all the chloride in the crystal of medaka T1r2a/T1r3LBD was replaced with bromide, and the diffraction data was collected using a wavelength of 0.9194 Å, close to the absorption edge of bromine, at BL41XU. The anomalous difference Fourier map derived from the data showed an eminent peak at the position where the presumed chloride ions were originally bound (Fig. 1(b)). These results indicate that the binding site was specific to halogen ions. Second, the diffraction data of the original crystal containing chloride was collected using a wavelength of 1.9 Å at BL41XU, as well as 2.7 Å at BL-1A at Photon Factory (Tsukuba, Japan). Although several kinds of light elements show similar levels of anomalous scattering,

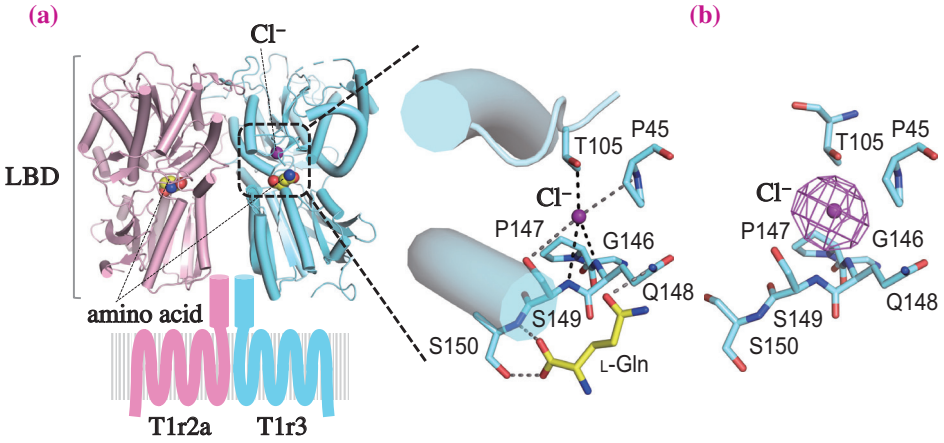


Fig. 1. The structures of the overall and the ligand-binding sites of T1r2a/T1r3 from medaka fish. **(a)** Schematic drawing of the overall structure of T1r2a/T1r3, in which the crystal structure of the ligand-binding domain (LBD) is shown (PDB ID 5X2M). The close-up view of the amino acid- and chloride ion-binding sites in T1r3 is shown in the right panel. **(b)** The anomalous difference Fourier map (5σ, magenta) derived from the diffraction data of the $Br^-$-substituted crystal is overlaid to the chloride ion-binding site in T1r3.

as chloride ions are present at this wavelength, a noticeable anomalous difference in the Fourier peak was observed at the presumed position of the chloride ion, while the other peaks were observed at the positions of the sulfur atoms in the protein. With these data taken together, we conclude that the unknown element bound to T1r3 is a chloride ion. Using a binding assay, we confirmed that chloride binding to T1r3 occurs not only in crystals, but also in solution.

We have addressed the physiological relevance of chloride binding to T1r3. Biophysical analyses showed that chloride binding induced a conformational change in medaka T1r2a/T1r3LBD in a manner similar to that of an amino acid (Fig. 2). This is likely because both the amino acid- and chloride ion-binding sites are located close to the interface between the two T1r subunits, which are known to reorient upon agonist binding during the activation of other class C GPCRs (Fig. 1(a)). Therefore, the surrounding structures may be affected by the binding or dissociation of these substances. Notably, based on the structure and amino acid sequences, the chloride ion binding site is likely to be conserved among T1r3s in many species, including mammals such as humans and mice. Indeed, we examined taste nerve responses in mice and found that chloride application induced taste nerve responses mediated by T1rs. These results indicate that chloride ions evoke taste sensations through sweet and umami receptors.

It has been reported that humans perceive low-concentration table salt solutions to be sweet [5]. The concentration range of sodium chloride-induced sweet taste is reportedly lower than those inducing salty taste evoked by sodium ions, but matches with those inducing the conformational change of medaka T1r2a/T1r3LBD and taste nerve responses in mice by chloride ions. Therefore, chloride sensation via T1r may occur even in humans (Fig. 2). The finding of chloride ion binding in T1r3 observed by X-ray crystallography led to the presumption that the sweet and umami receptors play a role in salt taste sensation, which is important for its intake at an appropriate amount to maintain body fluid homeostasis, and thus for human health.

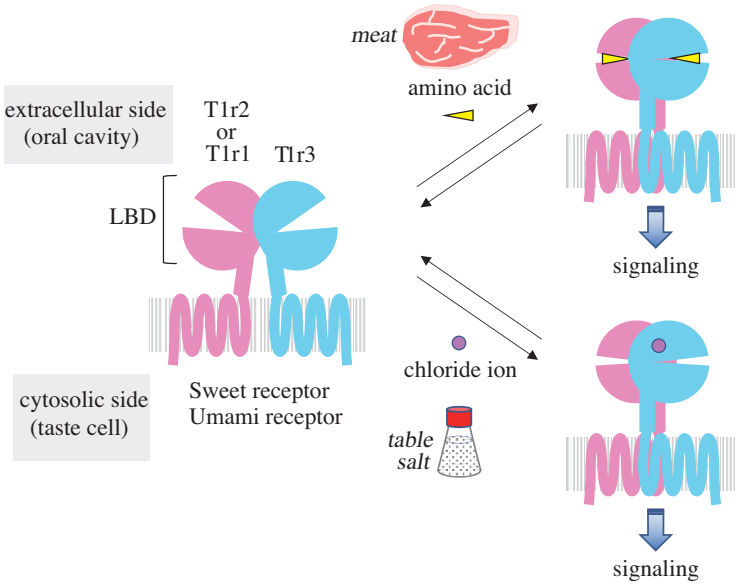


Fig. 2. Speculative mechanism of taste substance-induced responses by sweet and umami taste receptors. The schematic drawing shows the amino acid- (upper) and chloride ion- (lower) induced conformational change of the receptors and the resultant signal transductions.

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**References**

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