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The structure–function role of C-terminus in human bitter taste receptor T2R4 signaling

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ABSTRACT

Bitter taste, in humans, is sensed by 25 G protein-coupled receptors, referred to as bitter taste receptors (T2Rs). The diverse roles of T2Rs in various extraoral tissues have implicated them as a potential target for therapeutic intervention. Structure–function studies have provided insights into the role of transmembrane and loop regions in the activation mechanism of T2Rs. However, studies aimed at deciphering the role of their carboxyl-terminus (C-terminus) are limited. In this study, we identified a KLK/R motif in the C-terminus that is conserved in 19 of the 25 T2Rs. Using site-directed mutagenesis we studied the role of 16 residues in the C-terminus of T2R4. The C-terminus of T2R4 is polybasic with 6 of the 16 residues consisting of lysines, constituting two separate KK motifs. We analyzed the effect of the C-terminus mutations on plasma membrane trafficking, and characterized their function in response to the T2R4 agonist quinine. The majority of the mutants showed defective receptor trafficking with ≤50% expression on the cell surface. Interestingly, mutation of the distal Lys296 of the KLK motif in T2R4 resulted in constitutive activity. The K296A mutant displayed five-fold basal activity over wild type T2R4, while the conservative substitution K296R showed wild type characteristics. The Lys294, Leu295 and Lys296 of the KLK motif in T2R4 were found to perform crucial roles, both, in receptor trafficking and function. Results from this study provide unique mechanistic insights into the structure–function role of the C-terminus in T2R signaling.

1. Introduction

G protein-coupled receptors (GPCRs) form the largest and the most diverse superfamily of cell surface proteins [1]. The 25 human bitter taste receptors (T2Rs) belong to the GPCR superfamily. Like all GPCRs, T2Rs consist of seven transmembrane (TM) helices, three extracellular loops (ECLs) and three intracellular loops (ICLs), with a short extracellular N-terminus and an intracellular C-terminus (Fig. 1A). T2Rs are 290–333 amino acids long and are relatively divergent, showing ~30–70% amino acid identity [2]. The highly conserved amino acid residues and motifs found in most GPCRs generally have critical roles in the mechanism of receptor activation. For example, class A GPCRs have highly conserved motifs, the LxxxD motif in TM2, D/ERY motif in TM3, CWxP motif in TM6, and NPxxY motif in TM7 [3], which are absent in T2Rs. We have previously revealed 13 highly conserved residues and two conserved motifs in T2Rs, LxxxR in TM2 and LxxSL in TM5 [4,5]. These conserved residues and motifs bear no similarity to class A GPCR motifs, suggesting that T2Rs have a unique activation mechanism. Most of the structure–function studies in T2Rs are focused on determining the role of TM and loop region residues involved in T2R activation and ligand binding. The role of the C-terminus in T2R structure and function, however, remains poorly understood.

Previous studies have shown that the cytoplasmic C-terminal region of GPCRs plays an important role in receptor trafficking [6–9], intracellular signaling [8,10,11] and agonist-induced receptor internalization [12]. GPCR export from the endoplasmic reticulum (ER) represents the first step in intracellular trafficking of receptors and influences their cell-surface expression and function [13,14]. However, the definitive sequences involved in trafficking and/or signal transduction remain unclear. Sequence analysis of human GPCRs showed that basic residues are frequent in the membrane-proximal region of the C-terminus. Mutation of the C-terminal membrane-proximal basic residues (MPBRs) results in a marked reduction in the cell surface expression of multiple GPCRs [7,15–18], thus, suggesting that these residues are critically involved in mediating the anterograde trafficking of a broad range of membrane proteins, including GPCRs. The sense of taste has a significant impact on food selection, nutrition and health. It is, therefore, highly desirable to modulate bitter taste perception and T2Rs so that healthier bitter foods and medicines may be rendered more palatable. Studies in airway tissues demonstrate that endogenous T2Rs, upon activation with bitter tastants, lead to muscle relaxation

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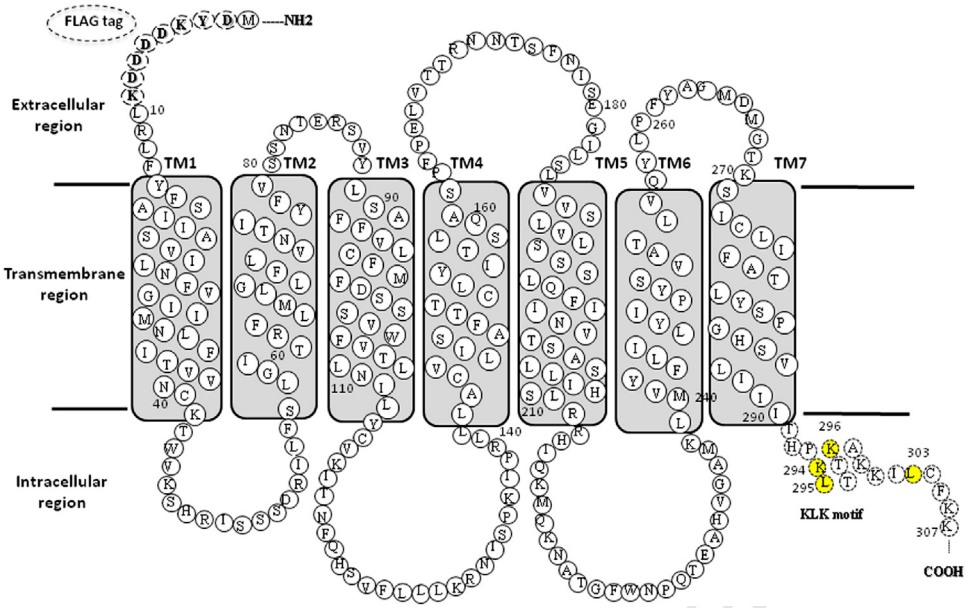
A



B

KLK/R motif

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hT2R1     GNPKLKQNAKKFLLHSKCCQ------------------ 299
hT2R3     GNSKLKQTFVVMLRCESGHLKPGSKGPIFS-------- 316
hT2R4     THPKLKTTAKKILCFKK--------------------- 299
hT2R5     GIPRVKQTCQKILWKTVCARRCWGP------------- 299
hT2R7     GNNKLRHASLKVI--WKVMSILKGRKFQQHKQI----- 318
hT2R8     LNNKLRQTFVRMLTCRKIACMI---------------- 309
hT2R9     GNSKLREAFLKML--RFVKCFLRRRKPFVP-------- 312
hT2R10    GNSKLKQASLRVL--QQLKCCEKRKNLRVT-------- 307
hT2R13    GNAKLRQA---------FLLVAAKVWAKR--------- 303
hT2R14    GNKKLRQASLSVLLWLRYMFKDGEPSGHKEFRESS--- 317
hT2R16    SSPTLKRILKGKC------------------------- 291
hT2R38    GNAKLRRAVMTILLWAQSSLKVRADHKADSRTLC---- 333
hT2R39    DNPGLRRAWKRLQLRLHLYPKEWTL------------- 338
hT2R40    GNPGLRRAWKRFQHQVPLYLKGQTL------------- 323
hT2R41    SNLKLRSVFSQLLLLARGFWVA---------------- 307
hT2R43    GNKKLKQTFLSVFWQMRYWVKGEKTSSP---------- 309
hT2R44    GNKKLKQTFLSVLRQVRYWVKGEKPSSP---------- 309
hT2R45    GNKKLKQTYLSVLWQMRY-------------------- 299
hT2R46    GNKKLKQTFLSVLWHVRYWVKGEKPSSS---------- 309
hT2R47    GNKKLKQIFLSVLRHVRYWVKDRSLRLHRFTRGALCVF 319
hT2R48    GSRKLKQTFLSVLWQMTR-------------------- 299
hT2R49    GNKTLKQTFLSVLWQVTCWAKGQNQSTP---------- 309
hT2R50    RTKKLKHTFLLILCQIRC-------------------- 299
hT2R55    GNSKLRQTAVRLL--WHLRNYTKTPNPLPL-------- 314
hT2R60    SNCRLRAVLKSRRSSRCGTP------------------ 318
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C

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Human     THPKLKTTAKKILCFKK----------------- 299
Chimpanzee THPKLKTTAKKILCFKK----------------- 299
Gorilla   THPKLKTTAKKILCFKK----------------- 299
Rhesus    THPKLKTTAKKILCFKK----------------- 299
Marmoset  THPKLKTTAKKILCFKK----------------- 299
Cattle    THPKLKTKAKNILCFSK----------------- 296
Dog       THPKLKTKAKKILCFNK----------------- 299
Mouse     THHKLKAKAKKIFCFYK----------------- 297
Rat       THHKLKAKAKKICCFYKLRDFVSN---------- 304
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Fig. 1. Amino acid sequence of the bitter taste receptor T2R4. A. Two-dimensional representation of the T2R4 amino acid sequence. It comprises a short extracellular N-terminus, seven transmembrane (TM) helices, three extracellular loops (ECLs), three intracellular loops (ICLs) and a short intracellular C-terminus. The C-terminus residues are shown in dashed circles. The conserved KLK motif and Leu303 are highlighted in yellow color. Also shown is the octapeptide FLAG-epitope at the N-terminus. B. Sequence alignment of the C-terminus of the 25 human T2Rs, without any FLAG-epitope tag at the N-terminus. T2R4 is shown in red, the conserved KLK/R motif is highlighted in green. C. Sequence alignment of C-terminus of T2R4 from different species. The sequences do not include the FLAG-epitope at their N-termini. Human T2R4 is shown in red, the KLK motif is highlighted in green, and the highly conserved amino acids are in blue color. Sequence alignment was done using the ClustalW algorithm. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this article.)

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