Pure Appl. Chem., Vol. 74, No. 7, pp. 1117–1123, 2002.

Homology-based model of the extracellular domain of the taste receptor T1R3*

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Abstract: The extracellular ligand binding domain of the sweet receptor T1R3 has been homology-modeled on the basis of the crystal structure of the metabotropic glutamate receptor (mGluR1). The region of the model that corresponds to the ligand binding site of mGluR1 has numerous polar and charged side-chains, consistent with expectations for a site that would respond to poly hydroxy compounds such as mono- and disaccharides. Docking studies show that proposed active conformations of the high-potency sweeteners neotame, superaspartame, and SC-45647 could interact favorably in this binding site, forming ion pairs or ionic hydrogen bonds with His-163, Glu-318, and His-407, in addition to hydrophobic interactions with numerous nonpolar side-chains.

INTRODUCTION

Several research groups have recently identified a likely sweet taste receptor, T1R3 [1–6]. This receptor is a member of the G protein coupled receptor superfamily, which encompasses many neurotransmitter and peptide hormone receptors, olfactory receptors, and visual receptors. These receptors have seven transmembrane alpha-helical segments, and they couple to a GTP-binding protein on the intracellular side of the cell membrane.

The human T1R3 has considerable sequence homology with metabotropic glutamate receptors (27 % sequence identity, 35 % sequence similarity). These glutamate receptors have a large (~500 amino acids) extracellular domain that dimerizes and binds the ligand, L-glutamate, as shown by the X-ray crystallographic studies of Kunishima et al. [7]. These workers expressed and crystallized the ligand binding domain (residues 33–522) of the type 1 metabotropic glutamate receptor (mGluR1), both in the presence and absence of glutamate. As shown schematically in Fig. 1, in the absence of ligand,

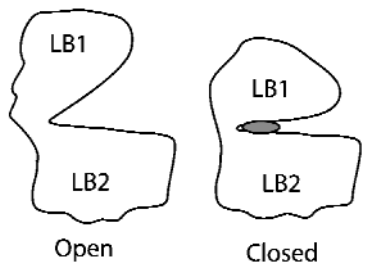

Fig. 1 Open and closed states of the monomeric extracellular domain of the mGluR1 receptor. LB1 = ligand binding lobe 1; LB2 = ligand binding lobe 2. Left, in the absence of glutamate, the two lobes of the extracellular domain exist in an open conformation. Right, upon binding of glutamate (represented by a filled ellipse), the two lobes adopt a closed conformation.

**Pure Appl. Chem.* Vol. 74, No. 7, 2002. A special topic issue on the science of sweeteners.
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each monomer adopts an open conformation; upon binding of glutamate, a closed conformation is induced.

Max et al. [1] showed a homology-based model of the extracellular domain of T1R3, based on the mGluR1 crystal structure [7]. In this paper, I describe an independently developed homology model of T1R3 (based on the same crystal structure) and, based on docking studies, propose ways in which high-potency sweeteners may interact with this receptor.

METHODS

The T1R3 sequence used in this work was the human sequence published by Max et al. [1]. The template for model construction was the crystal structure of mGluR1 extracellular domain [7] in the glutamate-bound form (Protein Data Bank code: 1EWK). Residue numbering is based on the mGluR1 crystal structure.

The sequences of mGluR1 (from the PDB file), human T1R3 [1], and mouse T1R3 [8] were initially aligned using the ClustalW program. Alignments were then manually adjusted to improve the overall alignment. In particular, the crystal structure of mGluR1 permitted identification of surface residues, which are the most likely locations for insertions and deletions. The final alignment is shown in Fig. 2.

Homology modeling was carried out using the Protein Design module of Quanta 98 (Accelrys Inc., San Diego, CA). The extracellular domain was modeled as a dimer in order to examine both the ligand binding site (which is fully contained in each monomer) and the dimer interface. First, residues were mutated according to the alignment in Fig. 2. Next, alternate conformations were found for sidechains making bad steric contacts. Deletions and insertions were carried out where needed. Polar hydrogen atoms were modeled in place. 1800 steps of minimization were carried out with the protein back-

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1EWK    MVRLLLIFFPMIFLEMSILPRMPDRKVLLAASSQRSVARMDGDVIIGALFSVHHQPPAEKVPERK
mt1r3   MPALAIMGLSLAAFLELGMGASLCLSQQFKAQGDYILGGLFPLGSTEEATLNQRTQPNSI--P---
ht1r3   MLGPAVLGLSLWALLHPGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPSS---P---

1EWK    CGEIREQYGIQRVEAMFHTLDKINADPVLLPNITLGSEIRDSCWHSSVALEQSIEFIRDSLISIRD
mt1r3   CNRFSP-LGLFLAMAMKMAVEEINNGSALLPGLRLGYDLFDTCSEPVVTMKSSLMF----LAKVGS
ht1r3   CTRFSS-NGLLWALAMKMAVEEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMF----LAKAGS

1EWK    EKDGLNRCLPDGQTLPPGRTKKPIAGVIGPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLSDKTLY
mt1r3   QSIA-AYC-NYTQYQPR------VLAVIGPHSSELALITGKFFSFFLMPQVSYSASMDRLSDRETF
ht1r3   RDIA-AYC-NYTQYQPR------VLAVIGPHSSELAMVTGKFFSFFLMPQVSYGASMELLSARETF

1EWK    KYFLRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESGMDAFKELAAQEGLCIAHSDKI-YS
mt1r3   PSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSIFSSLANARGICIAHEGLVPQH
ht1r3   PSFFRTVPSDRVQLTAAAELSQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIAHEGLVPLP

1EWK    NAGEKSFDRLLRKLRE-RLPKARVVVCFCEGMTVRGLLSAMRRLGVVGEFSLIGSDGWADRDEVIE
mt1r3   DTSGQQLGKVLDVLRQVNQSKVQVVVLFASARAVYSLFSYSIHHGLSPKV-WVASESWLTSDLVMT
ht1r3   RADDSRLGKVQDVLHQVNQSSVQVVLLFASVHAAHALFNYSISSRLSPKV-WVASEAWLTSDLVMG

1EWK    GYEVEANGGITIKLQS----PEVRSFDDYFLKLRLDTNTR-NPWFPEF--WQHRFQCRLPGHLLEN
mt1r3   LPNIARVGTVLGFLQRGALLPEFSHYVETHLALAADPAFC-ASLNAELDLEEHVMGQRCP--RCDD
ht1r3   LPGMAQMGTVLGFLQRGAQLHEFPQYVKTHLALATDPAFCSALGEREQGLEEDVVG----------

1EWK    PNFKKVCTGNESLEENYVQDSKMGFVINAIYAMAHGL-QNMHHAL-CPGHVGLCDAMKPIDGRKLL
mt1r3   IMLQNLSSG---LLQN-LSAGQLHHQIFATYAAVYSVAQALHNTLQC--NVSHCHVSEHVLPWQLL
ht1r3   QRCPQCDCI---TLQN-VSAGLNHHQTFSVYAAVYSVAQALHNTLQC--NASGCPAQDPVKPWQLL

1EWK    DFLIKSSFVGVSGEEVWFDEKGDAPGRYDIMNLQYTEANRYDYVHVGTWHEGVLNIDDYKIQ
mt1r3   ENMYNMSF-HARDLTLQFDAEGNVDMEYDLKMWVWQSPTPVLHT-VGTFN-GTLQLQQSKMY
ht1r3   ENMYNLTF-HVGGLPLRFDSSGNVDMEYDLKLWVWQGSVPRLHD-VGRFN-GSLRTERLKIR
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Fig. 2 Alignment of extracellular domain sequences of mGluR1 (label 1EWK), mouse T1R3 (label mt1r3), and human T1R3 (label ht1r3). **Boldface** indicates sequence similarity.

bone constrained, followed by 100 000 steps of molecular dynamics at 300 K with backbone constraints, in order to allow side-chains to find stable locations. Next followed 6000 steps of minimization, 50 000 steps of molecular dynamics, and 1300 steps of minimization with no constraints, in order to locate a stable backbone conformation.

Three high-potency sweeteners were investigated as possible ligands in this study. Neotame, superaspartame, and SC-45647 (Fig. 3) were docked in previously identified conformations [10]. I make the assumption that all three of these compounds are likely to bind to a common receptor site because they share a number of common structural features (a carboxylate group, two hydrophobic substituents, and three polar N–H groups). As shown previously [10], it is possible to identify superimposable conformations of these three ligands, which present a common pattern of a carboxylate group + two hydrophobics + two N–H groups to the receptor. Further, I make the assumption that the ligand binding site on the T1R3 receptor will be located in the cleft between the two lobes of the receptor, as is the case in mGluR1. Compounds **1–3** were docked manually into the homology-modeled T1R3 using the Quanta program; protein side-chains were rotated as necessary to alleviate unfavorable steric contacts; each receptor + ligand complex was subjected to 200–400 cycles of minimization.

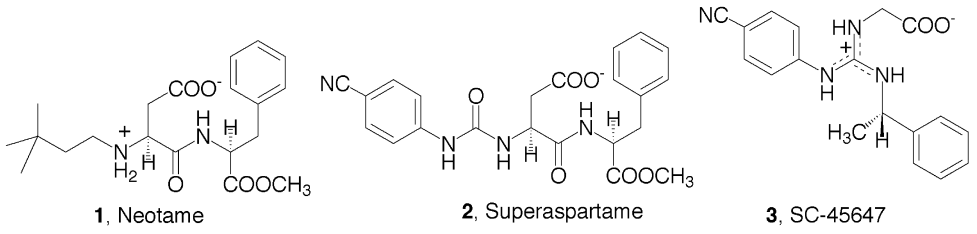

Fig. 3 Structures of high-potency sweeteners examined in this study: (**1**) neotame; (**2**) superaspartame; (**3**) SC-45647.

RESULTS AND DISCUSSION

Figure 4 shows a schematic representation of the dimeric receptor, as proposed by Kunishima et al. [7]. The only portion of the receptor that was crystallized (in the case of mGluR1) or modeled (in the case of T1R3) is the extracellular ligand binding domain (LB1 and LB2 in the figure).

The dimer interface is formed between LB1 and LB1' (the ' symbol indicates the second member of the dimer). In the case of mGluR1, almost the entire interface is formed by hydrophobic side-chain contacts (15 pairs of hydrophobic side-chain contacts); the two exceptions are a pair of hydrogen bonds between glutamine-170 and asparagine-173', and between glutamine-170' and asparagine-173. In the homology-modeled human T1R3, there are 19 pairs of hydrophobic side-chain contacts. Thus, the nature of the dimer interface appears to be quite highly conserved.

What is the nature of the cleft corresponding to the glutamate binding site in mGluR1? Figure 5 shows the alpha-carbon atoms of the T1R3 monomer model; the cleft opens to the right. It is apparent that the proposed binding site is highly polar. This is not surprising in light of the primary task of these receptors: to detect mono- and disaccharides, which are covered with hydroxyl groups. It is easy to picture one or more saccharides forming ionic and nonionic hydrogen bonds to the many polar residues on the bottom of LB1 and the top of LB2, causing the two lobes to come together. There are sufficient polar residues in this cleft so that many different mono- or disaccharides could interact favorably, consistent with the observation that many different polyhydroxy compounds taste sweet. The cleft is also large enough to accommodate more than one ligand at a time; if this receptor requires the presence of two or more sugar ligands in order to adopt the closed conformation, this would be consistent with the observation that, unlike most receptors, the sweet taste receptor is not activated until sugar concentrations reach about 0.1 molar.

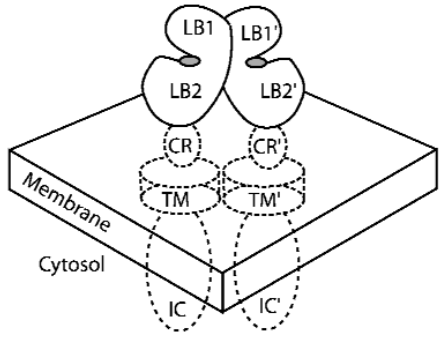

Fig. 4 Schematic representation of the receptor dimer (adapted from a drawing by Kunishima et al. [7]). Starting from the *N*-terminal, LB1 and LB2 are the two lobes of the ligand binding domain; CR is a cysteine-rich region; TM represents the 7 transmembrane helical segments; IC is the intracellular domain, which interacts with the GTP binding protein in the cytosol. The gray ellipses represent ligands bound in the receptor binding site.

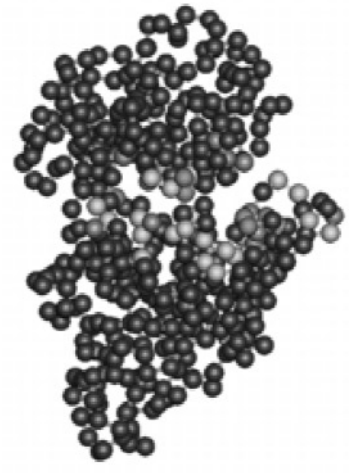

Fig. 5 T1R3 monomer model. Only alpha-carbons are shown. Within the cleft, polar and charged residues are shown as gray spheres; all others are shown as black spheres.

How could this receptor interact favorably with high-potency sweeteners such as neotame, superaspartame, and SC-45647? To investigate this, I docked each sweetener into the cleft, using previously identified conformations of these three sweeteners [10]. Since the carboxylate group is required for the sweet taste of these compounds, I began by identifying basic side-chains that could interact favorably with the carboxylate. I then looked for appropriately placed protein side-chains that could make favorable interactions with two or three of the polar N–H groups on each sweetener. Finally, I looked at ways in which the two hydrophobic substituents of each sweetener could be accommodated. Such a site exists in this model. His-163 and His-407, on the surface of LB1, are well situated to coordinate a carboxylate group. Glu-318, on the surface of LB2, is able to interact with a polar N–H group in each case. Finally, hydrophobic substituents on each sweetener are able to interact with hydrophobic side-chains in the proposed binding site. In the following paragraphs, I examine the binding interactions in detail.

Neotame

As shown in Fig. 6, the carboxylate group of neotame can coordinate well with His-163 and His-407. The NH_2^+ group of neotame forms an ion pair with Glu-318. The neohexyl substituent is in contact with Ala-90, Tyr-236, Ala-319, and the $–CH_2–$ group of Ser-292. The phenyl side-chain is enclosed by Leu-107, Phe-119, Val-155, and $–CH_2–$ groups of Arg-52 and Asn-74.

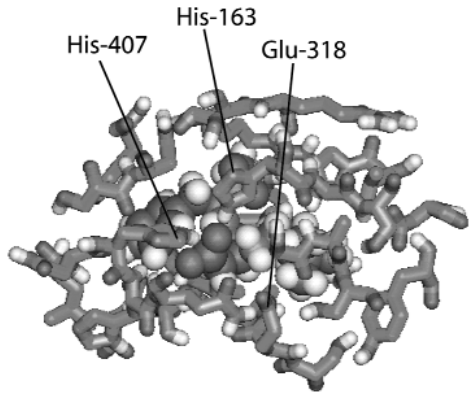

Fig. 6 Neotame docked into the proposed binding site of the T1R3 model. Receptor is shown as a stick structure, and neotame is shown as a space-filling structure.

Superaspartame

Figure 7 shows superaspartame docked into the same binding site. The carboxylate group interacts well with His-163 and His-407. The cyanophenyl-NH group pairs with Glu-318, and the other urea N–H pairs with the backbone carbonyl oxygen of Glu-318. The cyanophenyl ring is in contact with Tyr-236, Leu-262, Ala-319, and the $–CH_2–$ groups of Ser-164 and Ser-292. The phenylalanine side-chain of superaspartame is enclosed by Phe-71, Val-293, Leu-325, Leu-405, and $–CH_2–$ groups of Arg-52 and Asn-74.

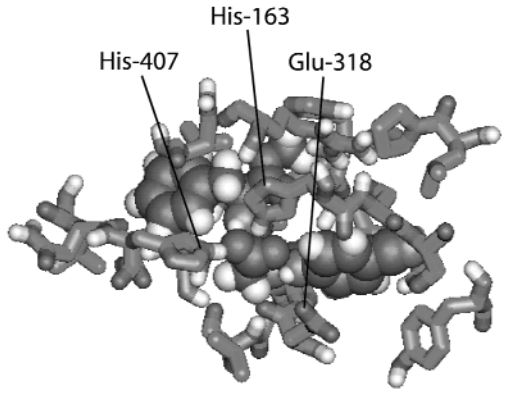

Fig. 7 Superaspartame docked into the proposed binding site of the T1R3 model. Receptor is shown as a stick structure, and superaspartame is shown as a space-filling structure.

SC-45647

In Fig. 8, we see SC-45647 docked into the binding site. Once again, the carboxylate group is positioned between His-163 and His-407. Glu-318 interacts with the $–CH_2–NH$ group. The cyanophenyl ring is in contact with Tyr-236, Ala-319, and the $–CH_2–$ groups of Ser-292 and Ser-317. The α-methyl-phenethyl side-chain is enclosed by Ala-55, Val-293, Leu-325, and $–CH_2–$ groups of Arg-52 and Asn-74.

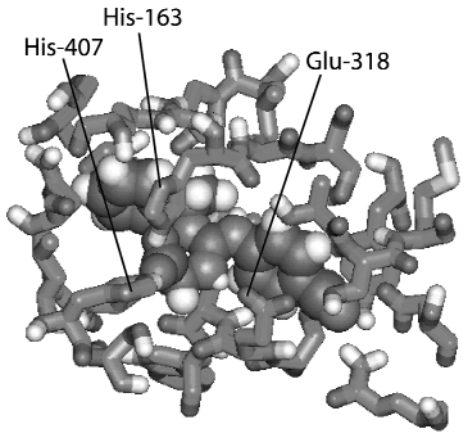

Fig. 8 SC-45647 docked into the proposed binding site of the T1R3 model. Receptor is shown as a stick structure, and SC-45647 is shown as a space-filling structure.

CONCLUSION

The sweet taste receptor T1R3 has recently been sequenced. In this paper I describe a homology-modeled three-dimensional model of the extracellular ligand binding domain, based on homology to the mGluR1, which has been crystallized with and without glutamate in the active site. It is possible to dock three structurally different high-potency sweeteners into this model in a consistent way. The model suggests single point mutations (e.g., to His-163, Glu-318, His-407), which could help to delineate the nature of the binding site. X-ray crystallographic studies of T1R3 co-crystallized with these and other high-potency sweeteners may someday shed further light on the nature of binding interactions.

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