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The Predicted 3D Structure of Bitter Taste Receptors, TAS2R38 Based on a BiHelix and SuperBiHelix Methodologies

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Abstract

TAS2R38 bitter taste receptors are seven-transmembrane (TM) domain G protein-coupled receptors (GPCRs) that can respond to bitter compounds such as Phenylthiocarbamide (PTC). We would like to understand the nature of the binding that is what aspect of the ligand interacting with the binding site leads to signal sent to the cortex. There are no direct determinations of the 3D structure of taste receptors; hence we use the new BiHelix and SuperBiHelix methods to predict the 3D structures of the TAS2R38 bitter taste receptors, which we chose because there is ample experimental data on how perception is related to the ligand and to mutations. These methods use a template to provide starting points for the structures and we tested four templates β1 Adrenergic Receptor (tβ1AR), β2 Adrenergic Receptor (hβ2AR), Bovine Rhodopsin and A_{2A} Adenosine Receptor ($hAA_{2A}R$). We found the tβ1AR template is more appropriate for structural simulation of the bitter taste receptors. We predicted 3D structures of for four haplotypes PAV, AVI, AAI and PVV of the TAS2R38 bitter receptors. Our results illustrate that the residue 262 is involved in the interhelical hydrogen bond network stabilizing the structure in tasters (PAV, AAI and PVV) while it is not in non-tasters (AVI). Thus the hydrogen bond interaction between TM3 or TM5 and TM6 may play a role in activating this GPCR.

Keywords: TAS2R38 bitter taste receptors, GPCRs, Structure prediction, BiHelix and SuperBiHelix, β1 Adrenergic Receptor

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1. Introduction

Bitter taste receptors are seven-transmembrane (TM) domain G protein-coupled receptors (GPCRs) quite distinct from such well studied GPCRs such as adrenocepters (e.g. β1 and β2 for which there are x-ray crystal structures). Thus this family of GPCRs is denoted as TAS2R. It is generally assumed that they warn us not to ingest potentially harmful compounds [1]. In humans, bitter taste is mediated by a family of ~ 30 bitter taste receptors (TAS2Rs) expressed in taste receptor cells [2-3]. Phenylthiocarbamide (PTC) is intensely bitter for some individuals, but is largely tasteless for others. This is attributed to 2 common forms of the TAS2R38 gene named for single-nucleotide polymorphisms (SNPs) resulting in 3 amino acid substitutions [4]. The three most common polymorphisms observed in TAS2R38 occur at

- amino acid (AA) position 49, where either pro or ala is encoded,
- AA position 262, where either ala or val is encoded, and
- AA position 296, where either val or ile is encoded,

This gives rise to two frequent haplotypes, PAV and AVI, plus less common haplotypes AAI, PVI, and AAV where

- PAV shows a strong response at micromolar concentrations for PTC, whereas
- AVI does not respond to these molecules.

Mutational analysis [5] determined that the amino acids at position 49 and 262 most affected the cellular response.

Several previous computational studies [6-8] used on structural modeling of bitter taste receptors based on structures build from homology to BovRh and considered binding of PTC or PROP to these bitter receptors. However only limit insights were provided about the molecular mechanism of bitter taste sensing. In particular these 3D structures [6-8], found that residue 262 points towards the TM7 of bitter taste receptors and does not interact with the PTC agonist, which is inconsistent with experiment data [5]..

To determine 3D structures that might address the difference between bitter taster structure and bitter non-taster structure and activation mechanism of bitter taste receptors, we used the BiHelix and SuperBiHelix methods developed recently at Caltech to predict the 3D structure of the bitter taste receptors haplotypes PAV (taster), AVI (nontaster), AAI (taster) and PVV (taster). We propose a preliminary explanation for activation of bitter taste receptors that is consistent with current experiment data [5] and which suggests new experiments that could be used to validate our structures.

2. Methods

2.1. Alignment and Homologize

We aligned the sequences of TAS2R38 receptors with β1 Adrenergic Receptor (tβ1AR), β2 Adrenergic Receptor (hβ2AR), Bovine Rhodopsin and A2A Adenosine Receptor ($hAA_{2A}R$), respectively. Then we mutated the residues of the templates to the aligned sequences of TAS2R38 receptors and generated the initial 3D structure of TAS2R38 receptors.

2.2. Construction of a template structure

The predicted TM domains of the protein were extracted to form 7 single helices, which were minimized using the dreiding force field [9] and then merged to form a 7 helix bundles that matches the template. Given the optimum helices to describe each of the 7 TM domains, they are placed into a 7-helix bundle using the x-ray template. Each experimental template has 42 degrees of freedom: x, y, z, θ, φ and η values for each of the seven TM helices ($6 \times 7 = 42$ total). The hydrophobic center is the residue that

crosses z = 0, which is defined as the plane that runs through the center of the lipid bilayer. It is either calculated from the protein's hydrophobic profile or by homology. The degrees of freedom that we optimize are the tilt angle of the helix θ, the sweep angle of the helix φ, and the rotation of the helix η around the helical axis.

2.3. BiHelix and SuperBiHelix

After minimization of helices, we consider all possible 7-helix bundles constructed by allowing each of the 7 helices to take on 12 orientations (30° increments) about their axes, which leads $12^7 = 35{,}000{,}000$ packings of the seven helices of the GPCR. The BiHelix procedure estimates the energies of these 35 million packings using a mean field constructed by considering the 12 sets of nearest neighbor bi-helix interactions, TM1-TM2, TM1-TM7, TM2-TM3, TM2-TM4, TM2-7, TM3-TM4, TM3-TM5, TM3-TM6, TM3-TM7, TM4-TM5, TM5-TM6 and TM6-TM7. Here we use SCREAM to optimize the side-chains for each case [10]. The details of BiHelix sampling method were described by Goddard [11].

For the optimum set of rotation angles (η) from step E, we now sample a range of tilts (θ, φ) simultaneous with η, to obtain the optimum 7-helix bundles. Again we consider the 12 pairs of strongly interacting helices but to account for the effect of tilts. The seven-helix bundle is partitioned into three quadhelix bundles, as shown in Fig. 1. The 2000 structures with the lowest energy for each quadhelix are selected by increasing energy. Finally, from each individual helical conformation list, the best 36 conformations for each helix are used to calculate the energy of $36^7 \approx 8 \times 10^{10}$ full bundles, and output the 1000 combinations estimated from this procedure to have the lowest energies.

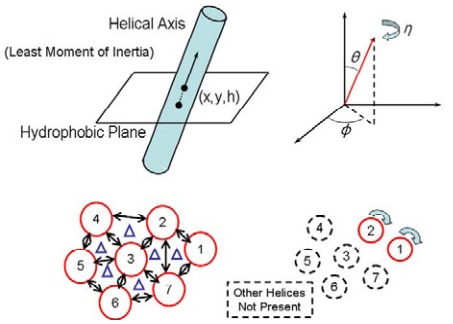

Fig. 1. The coordinates used to describe the orientation of the seven helices in a GPCR bundle. Double arrows connecting nearest neighbor helix pairs that are sampled independently in the BiHelix procedure. The BiHelix procedure is highlighted using helices 1 and 2 to show that when the conformations for this helix pair are sampled, other helices are not present.

3. Results and discussion

We shortened or lengthened the templates TMs and predicted the 3D structures of the TAS2R38 bitter receptors based on the four receptors templates tβ1AR, hβ2AR, Rhodopsin and $hAA_{2A}R$, respectively. The TMs sequence alignments between PAV and four templates (tβ1AR, hβ2AR, Rhodopsin and $hAA_{2A}R$) which are used to build the 3D structures by the homology modeling. After minimization, the initial structures combining the seven TM segments from the four templates (without loops and the eighth helices) were built. After BiHelix and ComBiHelix, the rotational angels of seven TMs were generated and top 2000 conformations of bitter taste receptors were obtained according to the total energy. Table 1 shows the best conformations of PAV receptor with the best total energy from BiHelix and ComBiHelix

results. The Helix-6 rotational angle of the structure based on tβ1AR template is larger than those based on other templates. The structure based on tβ1AR template has the lowest total energy compared with those based on other templates. Residue A262 points inside the bundles based on tβ1AR, while it points outside the bindles based on hβ2AR and towards TM7 of the structure based on Rhodopsin and $hAA_{2A}R$. It has been reported that the residue 262 in bitter taste receptor is very important for the bitter taste [5]. This suggests that a residue 262 is located inside the TMs bundles so that it can interact with the agonist. Thus we selected tβ1AR as the best template to predict the 3D structure of bitter taste receptors.

Therefore we constructed the structural models of the TAS2R38 bitter receptors (four haplotypes PAV, AVI, AAI and PVV) using the tβ1AR as a template based on the homology method. The ensemble of a few thousands conformations with different helical rotation angles η, tilt angle θ and sweeping angle φ were generated based on BiHelix and SuperBihelix technology [11]. We here discuss the details only for the best (the lowest total energy) predicted protein structures.

As shown in Table 2, BiHelix and SuperBiHelix results suggest that the helices of four variants have identical rotational angles except helix 5. The TAS2R38 bitter receptors lack some of the well-conserved motifs present in class A GPCRs. Thus we can expect that the TAS2R38 bitter receptors might have a different set of stabilizing interhelical hydrogen bonds from tβ1AR. The predicted 3D structures of four variants of the TAS2R38 bitter receptors are shown in Fig. 2, and the residues forming interhelical H-bonds are highlighted.

We find the interhelical hydrogen bonds between Y199 (5) and W108 (3), and between Y199 (5) and A262 (6) in PAV protein. An interhelical bond between W108 (3) and A(V)262 (6) exists in both AAI and PVV protein. These interhelical bonds don't exist in the AVI protein. The mutation of A262V and V296I results in a larger rotation angle of TM5 in AVI so that both the Y199 and W108 could not form H-bond with V262. Although the mutation of A262V exists in the PVV, the smaller rotation angle of TM5 can not cause the formation of TM5-TM6 hydrogen bond interaction. Thus the hydrogen bond interaction between TM3 or TM5 and TM6 may pass the signal to intracellular to activate receptor. In present, the docking of agonists and molecular dynamics simulation are undertaking for exploring the detailed activation mechanism of bitter taste receptors.

Table 1. BiHelix and ComBiHelix results for the PAV variation of bitter taste receptors in the different receptor templates.

Receptor Templates	Rotational Angle							Total Energy (Kcal/mol)
	H1	H2	H3	H4	H5	**H6**	H7	
tβ1AR	**30**	**330**	**60**	**90**	**180**	**270**	**30**	598.1
hβ2AR	30	330	60	120	330	**90**	270	754.5
Rhodopsin	0	0	60	150	330	**30**	330	997.3
hAA2AR	0	330	30	180	0	**30**	0	932.8

Table 2. BiHelix and ComBiHelix results for bitter taste receptors in the β1 adrenergic receptor template.

Receptor Variants	Rotational Angle							Total Energy (Kcal/mol)
	H1	H2	H3	H4	**H5**	H6	H7	
PAV	30	330	60	90	**180**	270	30	598.1
AVI	30	330	60	90	**330**	270	30	668.9
AAI	30	330	60	90	**240**	270	30	584.1
PVV	30	330	60	90	**240**	270	30	597.4

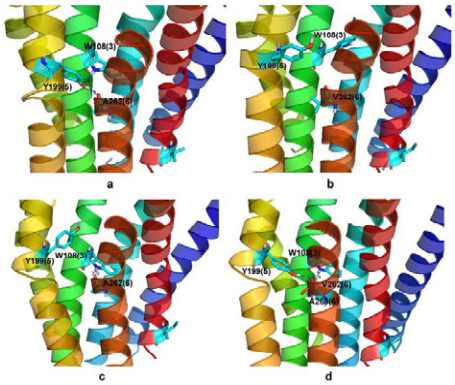

Fig. 2 Predicted 3D structures of bitter taste receptors (a) PAV, (b) AVI, (c) AAI and (d) PVV from SuperComBiHelix. (Residues forming interhelical H-bonds are highlighted here)

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