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Differences in (−)citronellal binding to various odorant receptors

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Abstract

To test the hypothesis that olfactory receptors (ORs) recognize different molecular features of odor molecules termed ''odotypes'', we studied receptor–ligand interactions of two human and two mouse ORs, recognizing (−)citronellal. Structurally similar receptors provide identical binding pockets (OLFR43, OR1A1, and OR1A2), and have comparable EC_{50} values. Other ORs with lower sequence identity bind (−)citronellal in a different way, leading to different EC_{50} values.

Keywords: Odorant receptor; Homology modelling; Receptor specificity; (−)Citronellal

Structures and functional mechanisms of G-protein coupled receptors (GPCRs) still remain a major goal in research. The crystal structure of bovine rhodopsin advanced our understanding of this huge class of membrane receptors [1] and up to now it is the only solved crystal structure of this important receptor class. However, evolutionary analyses revealed common structural motifs in the transmembrane helices of Class A GPCRs, which implies overall similar fold. Odorant receptors (ORs) constitute the largest group among Class A GPCRs, with more than 350 functional receptors in human and approximately 1000 in mouse [2,3]. These receptors discriminate between numerous chemically diverse molecules. Each odorant is recognized by different receptors and odorants bind to multiple receptors with distinct affinities and specificities leading to combinatorial odour coding [4]. Several research groups succeeded in identifying key residues involved in receptor specificity based on combinatorial approaches involving homology modelling and site-directed mutagenesis studies [5,6] as well as computational analyses [7,8]. Multiple sequence alignment analysis of ORs led to the identification of highly variable regions located in transmembrane helices 2–7 as well as in the second extracellular loop (EC2) [9], highlighting the importance of amino acid variability for ligand recognition. However, none of the previous methods addressed the question how different receptors recognize similar ligands, with similar or dissimilar efficacy. This question is hampered by the fact that despite intense efforts, only a small number of cognate receptor–ligand pairs have been identified (e.g. [10–13]). In the absence of a known 3D-structure, homology modelling methods provide a suitable means to study the interaction of odorants with different ORs. Here we will present a study focusing on the interaction of (−)citronellal with several human and mouse odorant receptors.

Materials and methods

Alignment. Sequences of OLFR43 (Accession No.: Q7TRX1), OR1A1 (Q9P1Q5), OR1A2 (Q9Y585) and OLFR49 (Q8VFB7) were taken from the UniProtKB/Swiss-Prot database [14]. Additionally, all human olfactory receptor sequences were downloaded from the HORDE database [15]. Redundant sequences were deleted, resulting in a multiple sequence alignment containing ~350 human receptors and selected mouse ORs.

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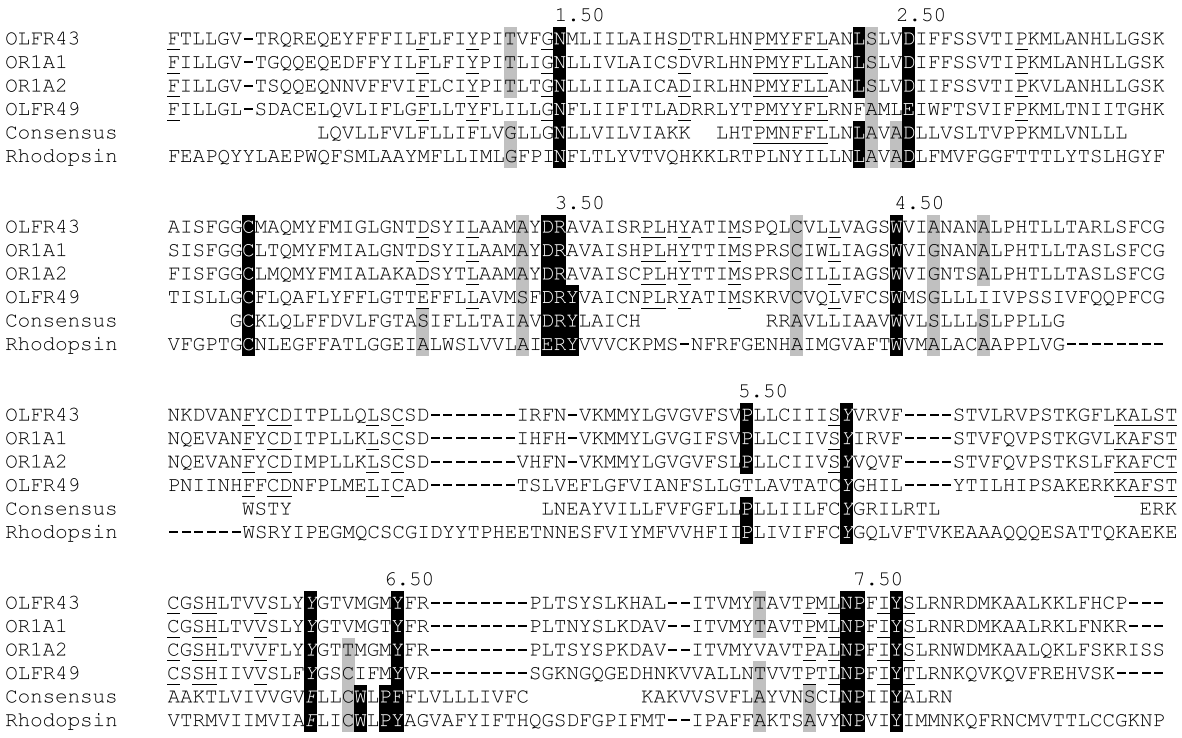

Fig. 1. Alignment of (−)citronellal recognizing ORs, together with a consensus sequence of Class A GPCRs and the bovine rhodopsin template. Numbers indicate the highly conserved sequence motifs (black boxes). Gray shaded are group conserved small and weakly polar residues, which are conserved in more than 80% of receptors [18]. Underlined are conserved residues in the olfactory receptor family.

Non-olfactory Class A receptors were taken from the GPCR database [16]. Initial alignments were generated using ClustalW [17], followed by manual improvements, to avoid indels in transmembrane helices and to assure correct alignment of conserved sequence motifs. Fig. 1 shows the alignment of ORs and bovine rhodopsin together with a Class A consensus sequence [18].

Model building. Initial models are based on the alignment shown in Fig. 1 using the Modeller software [19]. Structures were manually adjusted to optimize hydrophobic–hydrophobic contacts, hydrogen bonds and salt bridges. These models were then minimized using the OPLS2005 force field in Macromodel [20] with 1000 steps of steepest descent. This resulted in elimination of repulsive van der Waals contacts and improved the formation of hydrogen bonds and salt bridges. The stereochemistry of the models was checked using standard validation programs (PROCHECK [21] see Table 1).

Identification of binding sites. Putative binding site residues were identified using the 22 predicted highly variable amino acids [9], together with binding site information derived from mutagenesis studies [5,6]. This led to an "initial" putative binding pocket of 32 amino acids.

Ligand preparation and docking. We used GaussView [22] to generate the (−)citronellal starting structure and optimized the geometry with the Hartree–Fock, 3-21G basis set as implemented in Gaussian 03 [22]. GLIDE [20] standard parameters were used for docking and the highest ranking positions were collected and carefully analyzed.

Table 1
Model quality of ORs

Receptor	Organism	Procheck Φ/Ψ (%)	Reference
OR1A1	*H. sapiens*	91.0	[11]
OR1A2	*H. sapiens*	91.1	[11]
OLFR43	*M. musculus*	91.5	[10]
OLFR49	*M. musculus*	90.0	[10,24]

Results

Olfactory receptor models

The most crucial step in developing homology models is to correctly align target and template sequences. For distantly related proteins, such as ORs, which share approximately 20% sequence identity with bovine rhodopsin, the alignment remains the "bottleneck" of model quality. However, much of the ambiguity can be reduced using multiple sequence alignments. Helix 1–5 and 7–8 can be aligned using patterns of highly conserved motifs. Helix 6 is more difficult to align, because the highly conserved P6.50 is missing in ORs. The illustrated alignment was selected because it aligns the second highly conserved motif, F6.44 present in both families. Additionally, this way the group-conserved residue (A, C, G, S, T) at position 6.47, which is conserved in more than 80% of Class A GPCRs is positioned correctly (see Fig. 1).

Identification of olfactory receptor specific structural features

It is accepted that different receptor subtypes may have evolved quite specific structural and functional features, reflected in the specific sequence signatures of each subtype. Multiple sequence alignments of ORs reveal such a signature in helix 3: a highly conserved D (E) at position 3.39, which can form a hydrogen bond to the side-chain of

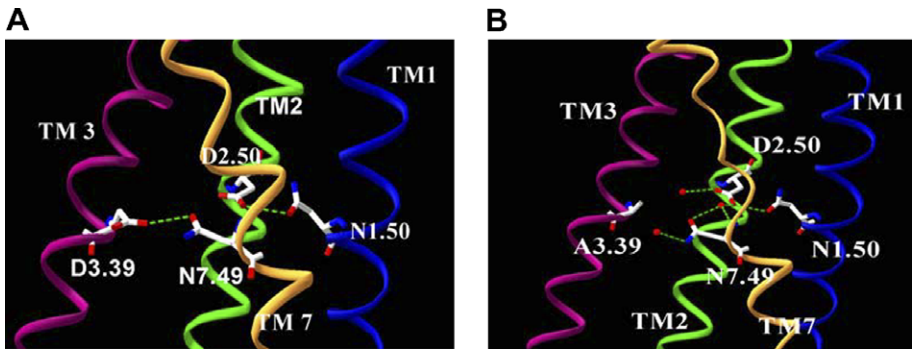

Fig. 2. Comparison of specific structural features in ORs with bovine rhodopsin. (A) A specific signature motif of olfactory receptors, revealed by multiple sequence alignments, is shown. Helix 3 contains a highly conserved negatively charged D(E) at position 3.39, which facilitates hydrogen bonding to the highly conserved N7.49 of helix 7. The latter residue is also conserved in bovine rhodopsin as can be seen in (B). Additionally, the hydrogen bond network between N1.50, D2.50 and N7.49, which involves several water molecules, is shown. This hydrogen bonding pattern is also conserved in ORs. It is reasonable to assume similar or related water mediated helix–helix contacts for olfactory receptors.

N7.49 of helix 7. Most other Class A receptors contain a small or weakly polar residue at this position (see Table 1 and Fig. 2A,B). Another difference is observed in helix 6, where the conserved CWXP motif, which is thought to play a critical role in receptor activation, is missing. Therefore, it can be assumed that activation of ORs is essentially different. Helix 6 of bovine rhodopsin adopts an unusual strong bend angle of 35°. Since the proline residue is not conserved in ORs, we inspected the helix for other possible hinge forming residues. Likely candidates are serines or threonines, which have been shown to affect backbone helix conformation via formation of intrahelical hydrogen bonds between their Oγ atoms and the i-3 or i-4 carbonyl oxygen of the helix backbone [23]. All ORs studied in this paper contain several serine, threonine and glycine residues in helix 6, which might act as flexible hinge points in these receptors.

Putative binding site for (−)citronellal

As a starting point all 32 putative binding site residues were included in docking studies performed with GLIDE. The highest ranking results were collected and carefully analyzed. In agreement with previous studies [9], we reasoned that identical residues are involved in (−)citronellal recognition for ortholog receptors, especially if they exhibit identical efficacy (EC_{50} values for OLFR43: 2.1 ± 0.2 μM vs 2.2 ± 0.2 μM for OR1A1 [10,11]).

Binding site comparison of these two receptors revealed identical amino acids involved in ligand binding. Only V205 of the mouse receptor is replaced with the functionally similar isoleucine (see Table 2). Studies by Krautwurst et al. [11] revealed that a paralog of OR1A1, OR1A2 also responds to (−)citronellal with a slightly modified EC_{50} value: 2.4 ± 0.4 μM. Therefore, we examined the amino acid differences in the putative binding site. Overall, the active site seems quite well conserved. However, there are several minor structural changes in the binding site, resulting from conservative replacements. V254 of helix 6 is replaced by the isosteric threonine and T277 of helix 7 is replaced by a valine. Interestingly, the position of the latter two residues is interchanged. Next we addressed the question of similar or dissimilar structural features in the binding site of other (−)citronellal recognizing receptors. We selected OLFR49, because the EC_{50} value is not too different from the above described receptors (EC_{50}: 3.3 ± 0.4 μM, [10]). The sequence identity between OLFR43 and OLFR49 of 37.3% is quite low. It is therefore reasonable to assume different binding site features. Indeed, significant differences were found and dissimilar receptor–

Table 2
Residues within 6.5 Å of putative (−)citronellal binding site

	OLFR43	OR1A1	OR1A2	OLFR49
Helix II	M81	—	V81	F71
Helix III	Q100, M101, M104, I105, N109, D111, S112	—	K109	Y102, F103, G106, T107
Helix IV	N155	—	S155	L153, I154
EC2	P183, L194, S188, C189, S190, D191	—	—	F176, V193,
Helix V	G204, V205,	I205	—	G197, F198, I200, A201, S204, L205,
Helix VI	Y251, V254, M255,Y258, F259	—	T254	Y250, C253, I254, Y257, V258
Helix VII	V274, T277, A278, T280, P281	—	V277	T278

Putative binding site residues, as identified by docking analysis, based on the concept of hypervariable residues. For OR1A1 and OR1A2 only residues different from the OLFR43 sequence are shown. While the binding site for OLFR43, OR1A1 and OR1A2 is quite similar, significant differences are observed in OLFR49. For example in helix IV there are polar amino acids in the former three receptors, possibly involved in ligand recognition, while OLFR49 contains only hydrophobic residues (boxed gray), in this region. Putative hydrogen bond donors are underlined.

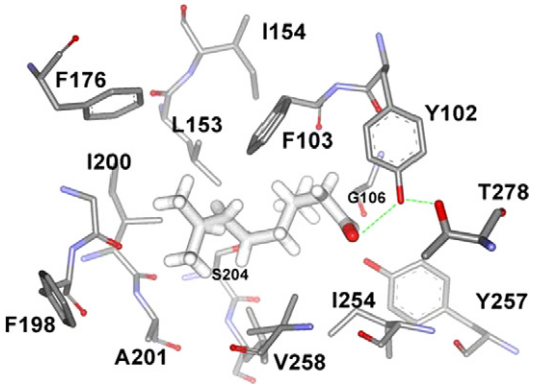

Fig. 3. Details of the OLFR49 binding site. Putative orientation of (−)citronellal in the OLFR49 binding pocket. Selected hydrogen bonds are shown in green. In agreement with previous studies [5] the pocket is formed predominantly by hydrophobic residues. The only polar interactions are between Y102 and T278 from helix 3 and 7, which may act as hydrogen donors. For clarity reasons, not all residues listed in Table 2 are shown.

ligand interactions were revealed by docking simulations. The putative orientation of (−)citronellal in OLFR49 is shown in Fig. 3. A key difference to OLFR43 is the location and nature of polar residues. As can be seen in Fig. 3 the aldehyde group of (−)citronellal is connected to Y102 of helix 3 and T278 in OLFR49. In contrast, OLFR43 as well as the two human receptors possess a methionine at the equivalent position. Docking studies of OLFR43 propose different hydrogen bond connections to the aldehyde group in this receptor (See Table 2).

Discussion

Ortholog receptors from different species recognize similar ligands. In the case of OLFR43 and its human ortholog OR1A1 this has been confirmed experimentally for the odorant (−)citronellal. Our studies reveal identical interactions of (−)citronellal with OLFR43 and OR1A1. The putative binding site differs at one position only, where a conservative valine is replaced by an isoleucine. According to our docking predictions, this residue is not directly involved in ligand–receptor interaction. Furthermore, our studies suggest only slight modifications in the binding site of OR1A2, a paralog of OR1A1 with 83.5% sequence identity. Differences include substitutions of T254 to a valine and V277 to a threonine which are presumably involved in van der Waals interactions with (−)citronellal. These slight modifications, together with more significant changes in the vicinity of the ligand binding site could be responsible for the slight increase in EC_{50} value (see Table 2). This is in agreement with experiments [5], which showed that conservative changes in the binding site result in subtle efficacy changes. Comparison of these results with OLFR49, which is only distantly related to the above mentioned receptors, strengthens the hypothesis that each odorant molecule possesses different "odotypes", resulting in different interactions with different receptors. Our docking simulations reveal that the aldehyde group interacts with different amino acids in OLFR43 and OLFR49 (see Table 2 and Fig. 3). These two receptors also exhibit different specificity for functional groups, because OLFR43 responds also to alcohols (e.g. (−)citronellol, geraniol), while OLFR49 does not recognize (−)citronellol [24]. It seems that ORs exhibit various degrees of specificity. Such a scenario has been proposed for olfactory receptor I7 from *Rattus norvegicus*, where the aldehyde group was shown to be of critical importance for receptor activation [13]. In contrast the human odorant receptor OR1G1 does not discriminate between functional groups [12] further strengthening the hypothesis that receptors recognize different features of odour molecules.

Our models also reveal an OR specific structure feature involving helix 3 and 7, which could be important for stabilizing the receptor in the inactive form. The functional importance of this motif can be tested experimentally and will help understanding receptor activation, which is expected to be different in ORs, because of the lack of a otherwise highly conserved sequence motif in helix 6, which is thought to play a key role in activation. In addition, a more detailed knowledge of receptor activation will improve receptor–ligand interactions studies, because up to now all models using bovine rhodopsin as template are describing inactive receptors.

Recently a new paper was published dealing with structural determinants of odorant recognition of the human ORs OR1A1 and OR1A2. The experimentally confirmed binding site residues are in good agreement with our independent findings, validating our predictions [25].

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