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Structural determinants of odorant recognition by the human olfactory receptors OR1A1 and OR1A2

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Abstract

An interaction of odorants with olfactory receptors is thought to be the initial step in odorant detection. However, ligands have been reported for only 6 out of 380 human olfactory receptors, with their structural determinants of odorant recognition just beginning to emerge. Guided by the notion that amino acid positions that interact with specific odorants would be conserved in orthologs, but variable in paralogs, and based on the prediction of a set of 22 of such amino acid positions, we have combined site-directed mutagenesis, rhodopsin-based homology modelling, and functional expression in HeLa/Olf cells of receptors OR1A1 and OR1A2. We found that (i) their odorant profiles are centred around citronellic terpenoid structures, (ii) two evolutionary conserved amino acid residues in transmembrane domain 3 are necessary for the responsiveness of OR1A1 and the mouse ortholog Olfr43 to (*S*)-(−)-citronellol, (iii) changes at these two positions are sufficient to account for the differential (*S*)-(−)-citronellol responsiveness of the paralogs OR1A1 and OR1A2, and (iv) the interaction sites for (*S*)-(−)-citronellal and (*S*)-(−)-citronellol differ in both human receptors. Our results show that the orientation of odorants within a homology modelling-derived binding pocket of olfactory receptor orthologs is defined by evolutionary conserved amino acid positions.

Keywords: Olfactory receptors; Human; Orthologs; Mutation; Docking model; Odorant

1. Introduction

Olfactory receptors in humans are encoded by about 380 genes (Malnic et al., 2004; Niimura and Nei, 2003; Zozulya et al., 2001). This olfactory receptor (OR) repertoire, is a bio-molecular interface between the chemical outside world and the sensory neurons of the olfactory epithelium (OE), and enables humans to detect, discriminate, categorize, and qualitatively and quantitatively evaluate a multitude of chemically diverse odorants. For example, about 8000 volatiles have been identified just in food (Grosch, 2001). While each OR may recognize several odorants (Malnic et al., 1999), their ligand specificity is, nevertheless, defined by efficacy-ranking odorant profiles (Katada et al., 2005; Shirokova et al., 2005).

If the structure of an OR determines its function, which then are the amino acid positions within OR that odorants interact with, and how does that translate into differences in their odorant profiles? Over the last decade, several groups have employed computational methods, based on a structure of rhodopsin (Palczewski et al., 2000), on the few OR with known ligands, to predict single amino acids that may be involved in odorant interaction (Afshar et al., 1998; Araneda et al., 2004; Floriano et al., 2000; Hall et al., 2004; Singer, 2000; Singer et al., 1996; Vaidehi et al., 2002); for review see Lai et al.

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(2005), Man et al. (2004), Olender et al. (2004). Rather than single amino acid positions, one study proposed 86 amino acid motives within the superfamily of OR that, in a combinatorial way, may enable differential odorant detection (Liu et al., 2003). In contrast, in a comprehensive study, based on ortholog/paralog comparisons among human and mouse OR. Man et al. (2004) predicted a set of 22 evolutionary conserved and putative odorant-interacting amino acid positions. Recently, 8 of these 22 amino acid positions were, however, identified to overlap with the mapped interaction sites for two single odorants within two mouse OR (Abaffy et al., 2006; Katada et al., 2005). A first mutational study to correlate single amino acid differences within OR-I7 orthologs from mouse and rat with differences in their preference towards an activation by heptanal or octanal was described earlier (Krautwurst et al., 1998). Two hundred human OR display >80% amino acid identity with mouse OR (Zhang and Firestein, 2002), and, thus, are potentially encoded by orthologous OR genes. For example, OR1A1 is the human ortholog to mouse Olfr43 (Glusman et al., 2000; Lapidot et al., 2001), for which we have recently described an odorant response pattern (Shirokova et al., 2005).

Here, we have de-orphaned OR1A1 and its paralog OR1A2 in HeLa/Olf cells. By rhodopsin-based homology modelling, site-directed mutagenesis, and functional expression of wild-type and mutant OR, we identified evolutionary conserved amino acid residues to be necessary and sufficient for the specific responsiveness of OR1A1 and OR1A2 to their odorants.

2. Methods

2.1. Molecular cloning

We amplified the full-length coding regions of OR1A1 (NM_014565), and OR1A2 (NM_012352) from human (HeLa) genomic DNA by polymerase chain reaction (PCR) with Pfu (Promega), or PfuUltra (Stratagene) using gene-specific primers (OR1A1: CAGGCAATTGATGAG GGAAAATAACCAGTCCTCTAC; CACTAGCGGCC GCTTACGAGGAGATTCTCTTGTTGAAGAG; OR1A2: GTCAGAATTCATGAAGAAAGAAAATCAATCCTT TAACCTG; GTCTAGCGGCCGCCTATGAGGAGAT TCTCTTGCTG). Amplicons were subcloned EcoRI/NotI or MfeI/NotI into the expression vector pi2-dk(rt39), which provides the first 39 amino acids of the bovine rhodopsin (rho-tag(39)) as a N-terminal tag for all full-length OR. Amino acid mutations in OR were achieved by the PCR-based QuickChange method (Stratagene). The identities of all subcloned wild-type (wt) and mutated OR coding region amplicons were verified by sequencing (MWG, Ebersberg, and UKEHH, Hamburg). Cell culture and transient DNA transfection were performed as reported previously (Shirokova et al., 2005).

2.2. Reverse transcriptase (RT)-PCR

Total RNA was prepared from a surgical biopsy of human main olfactory epithelium, taken from the dorsal region of the superior nasal concha, using Trizol (Invitrogen). Total RNA was treated with DNaseI (Invitrogen), and 50 ng served as template for each reverse transcriptase-PCR (One Step RT-PCR, Quiagen), using gene-specific primers for OR1A1 (CATTGTCCTAGCCATTT GCTCTGATG; CTTGAGCACGCCCTTGGTGGAAG) and OR1A2 (CATCTTGGCCATCTGTGCTGACATTC; CTTTGAATAGACTCTTGGTAGATGG).

2.3. Ca^{2+}-FLIPR assay

FLIPR assays (Molecular Devices) and data analyses were performed as described previously (Shirokova et al., 2005). In short, cells were loaded with 4 μM FLUO-4/AM and 0.04% Pluronic F-127 (both Molecular Probes) in Hepes-buffered saline (HBS) with 20 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (Hepes) and 2.5 mM probenecid. After loading, cells were washed twice with HBS by an automated plate washer (Denley Cellwash, Labsystems) and transferred to the FLIPR. EC_{50} values and curves were derived from fitting the function $f(x) = (a-d)/(1+(x/C)^{nH}) + d$ to the data by nonlinear regression with a, minimum; d, maximum; C, EC_{50}, and nH, Hill coefficient.

2.4. [^{35}S]GTPγS binding assay

[^{35}S]GTPγS binding assays were performed as described previously (Shirokova et al., 2005) with modifications that include a G-protein immunoprecipitation step (Milligan, 2003). HeLa/Olf cells were grown in a 10-cm dish and transfected with 6 μg of receptor DNA, or empty vector, using PolyFect (Quiagen). For the binding reaction, cell membrane protein (~50 μg) was incubated for 15 min at 37 °C in a binding buffer (10 mM Hepes, pH 7.4, 3 mM $MgCl_2$, and 50 mM NaCl) that included 0.05 nM [^{35}S]GTPγS and 3 μM GDP in a total volume of 100 μl. Basal condition was determined in the absence of agonist. Parallel assays containing unlabeled GTPγS (10 μM) were used to define non-specific binding. The reactions were stopped by addition of ice-cold binding buffer and centrifuged at 16,000g at 4 °C for 30 min to pellet the protein. After centrifugation, the pelleted protein was resuspended in solubilization buffer (10 mM Hepes, pH 7.4, 50 mM NaCl, 5 mM ethylenediaminetetraacetic acid [EDTA], and 0.5% Triton X) that included 1 μg anti-Gαs/αolf (rabbit, Calbiochem), and 20 μl protein A-conjugated agarose beads, and rotated overnight at 4 °C. The beads were washed three times with solubilization buffer, and the bound radioactivity was measured in a liquid scintillation counter. Under these conditions, non-specific binding was typically <10% of the total binding. The non-specific binding was substracted, and the basal value was set at 100%.