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Research article

The human olfactory receptor 17-40: Requisites for fitting into the binding pocket

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ABSTRACT

To gain structural insight on the interactions between odorants and the human olfactory receptor, we did homology modelling of the receptor structure, followed by molecular docking simulation with ligands. Molecular dynamics simulation on the structures resulting from docking served to estimate the binding free energy of the various odorant families. A correlation with the odorous properties of the ligands is proposed. We also investigated which residues were involved in the binding of a set of properly synthesised ligands and which were required for fitting inside the binding pocket. Olfactive stimulation of the olfactory receptor with odorous molecules was also investigated, using calcium imaging or electrophysiological recordings.

1. Introduction

Olfactory receptors (ORs) are members of the rhodopsin-like class of G-protein coupled receptors (GPCRs) and have the characteristic seven-transmembrane (TM) helical motif (Buck, 2005). The sequence characteristics of ORs are not shared by other GPCRs, and their marked diversity is consistent with the ability to recognise structurally different odorants (Buck and Axel, 1991). Each OR interacts with a variety of structurally related odor molecules. Bioinformatic analyses of genome sequence databases indicate that there are nearly 1000 functional ORs in mice (Godfrey et al., 2004), and about 350 in humans (Malnic et al., 2004). The combinatorial nature of odor coding explains how humans can detect and distinguish a very large number of odorants using "only" 350 receptors.

Studies on interactions between ORs and odorous molecules in olfactory neurons have helped understand the molecular basis of the olfactory code. The genes encoding ORs have been expressed in different heterologous systems (Matsunami, 2005; Wetzel et al., 1999; Monastyrskaia et al., 1998), in cell lines with a neuronal phenotype (Gimelbrant et al., 2001), or directly in the olfactory epithelium (Araneda et al., 2000). Isolated olfactory neurons (ORNs) have also been tested for their ability to respond to an odorous stimulus (Duchamp-Viret et al., 2000; Kaluza and Breer, 2000; Malnic et al., 1999; Touhara et al., 1999) but in view of the large number of potential ligands and the lack of a rapid and simple screening test, these studies have been limited to only a few ORs.

Thus, to understand the neural basis of odor discrimination the interactions of odorant molecules with their OR proteins must be modelled. When ORs were identified Buck and Axel (1991) noted that non-conservative changes were commonly observed within blocks of residues in TM regions 3, 4 and 5; these might indicate the sites of direct contact with odorous ligands. This was in fact confirmed by molecular models and sequence analyses which also predicted a binding pocket composed of specific residues in TMs 3–6 (Abaffy et al., 2007; Doszczak et al., 2007; Singer, 2000). A further study of putative binding pockets identified pairs of sequence positions where residues remain conserved or mutated in tandem, suggesting structural changes of functional importance. Molecular modelling studies indicated that several amino acid residues might be involved in ligand binding. Two of these positions were dominated by histidine residues, which might preferentially interact with aldehyde groups (Zhao et al., 1998), with important roles in ligand binding, thus conferring specificity on mammalian ORs (Singer, 2000).

The rat OR-I7 was the first mammalian OR for which a specific ligand (octanal) was identified (Zhao et al., 1998). It has been investigated further (Araneda et al., 2000) in studies involving a wide range of odorous molecules. These showed that each OR can recognise several structurally related odorants and that the same odorant is recognised by a number of ORs. Consequently, odors are distinguished on the basis of different combinatorial patterns of response elicited by the ORs (Malnic et al., 1999; Touhara et al., 1999).

OR 17-40 was the first human receptor to be cloned, expressed and functionally characterized. Helional was identified as its most effective ligand (Wetzel et al., 1999; Hatt et al., 1999). Results reported by Singer (2000) for OR-I7 and Hatt et al. (1999) for OR 17-40 were successfully reproduced by Levasseur et al. (2003),

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who expressed both rat OR-I7 and human OR 17-40 in mammalian cells to investigate their ligand-binding specificities. The cells expressing recombinant OR-I7 exclusively responded to aldehydes, namely heptanal, octanal and nonanal, in line with previous reports (Araneda et al., 2000; Glusman et al., 1996), where octanal was the main ligand for the rat OR-I7. These results and those of Levasseur et al. (2003) are in fact complementary. In the case of the human OR 17-40, mainly helional among other odorants tested elicited a response from the cells expressing the receptor (Jacquier et al., 2006; Wetzel et al., 1999).

To explore the mechanism of interaction of established odorants we: (i) built a 3D model of this human receptor, (ii) identified the amino acids lining the putative binding pocket and (iii) searched for new putative ligands for OR 17-40.

We synthesised and tested a set of odorous compounds in which the carbonyl group was replaced by a vinyl group. This modification was introduced in the molecule of helional (the best ligand for OR 17-40) and heliotropyl acetone (the second-best ligand), to verify how the carbonyl group influenced the OR interaction. We also did docking simulations with hydroxycitronellal, another interesting floral odor molecule (like helional) with a different molecular structure, that we had studied before in our laboratory (Anselmi et al., 1996).

2. Materials and methods

2.1. OR 17-40 molecular modelling

The OR 17-40 model was built up based on its alignment with the crystal structures of bovine rhodopsin 1GZM (Li et al., 2004) and human β2 adrenoceptor 2R4S (Rasmussen et al., 2007) using DeepView software (Guex and Peitsch, 1997). The OR 17-40 model was subjected to 900 cycles of energy minimization using the GROMACS v3.0 software and force field (Lindahl et al., 2001).

2.2. Molecular docking

Docking was done using AutoDock 3.0.5 (Morris et al., 1998) and a genetic algorithm, with a "local search" according to the Solis & Wets algorithm (Belew and Mitchell, 1996). A $54 \times 54 \times 54$ three-dimensional grid was created, generating a cube measuring 20.25 Å each side. The protein inside the membrane or in contact with it was excluded because it is inaccessible to ligands in the biological system.

The docking process was repeated 100 times for each odorous molecule so to permit statistical sampling from which families with inner root mean square deviation (RMSD) of 1.0 Å or less were collected. For hydroxycitronellal (**6**) and its analogues **7**, **8**, **9** and **10**, clustering with 1.0 Å tolerance produced more families than in previous cases, all closely related because of the greater molecular flexibility which generates larger uncertainties in conformation. Therefore, to obtain the dominant structure we raised the clustering tolerance to 2.0 Å.

For the genetic algorithm we applied the following parameters, reported to be optimal for small numbers of torsions (Heteney and van der Spoel, 2002): 50 subjects in the population, 250,000 energy evaluations, 270,000 generations, one subject survives for each generation, 2% mutation probability, 80% cross-over probability.

2.3. Binding free energy

Molecular dynamics of all ligands, free and complexed, were investigated using GROMOS force field (and GROMACS software) (Lindahl et al., 2001). The free or complexed ligand was placed at the centre of a periodic cubic box filled with respectively 1183 or 8196 water molecules, and 8 Cl^- ions to ensure a net charge of 0. To relax the configuration of the whole system steep descent minimization was carried out. After minimization of the system, equilibrium was reached using position restraint dynamics, where the protein atoms are blocked in their position while water molecules are free to move. Over the whole 100 ps the temperature (T) was raised stepwise from 0 K to 300 K in 2-fs steps. The bath temperature was 300 K with 1 atm constant pressure, using a pressure relaxing time of 0.5 ps in both cases. Bond lengths were constrained to an ideal value, using LINCS algorithm (Lindahl et al., 2001).

2.4. Synthesis of odorants

2.4.1. General method and materials

Helional and heliotropyl acetone were kindly supplied by IFF (Trezzano sul Naviglio, Milan), hydroxycitronellal by Cerizza (Cormano, Milan). All other materials used were from Sigma–Aldrich (Milan).

The structures of all compounds were established by ^{1}H NMR spectroscopy. To the best of our knowledge compounds **3**, **7**, **8**, **10** and **15** have not been previously described and their NMR spectra are now reported. ^{1}HNMR spectra were recorded on a 200 MHz Bruker AC-200 in $CDCl_3$. Chemical shifts (δ) are indicated in ppm with reference to tetramethylsilane as internal standard.

2.4.2. Synthesis of 5-(2-methyl-but-3-enyl)-benzo[1,3]dioxole (*3*), 5-(3-methyl-but-3-enyl)-benzo[1,3] dioxole (*4*), 2,6-dimethyl-8-nonen-2-ol (*7*) and 4,8,8-trimethyl-1-nonene (*10*)

These odorants were prepared by Wittig reaction between methyltriphenyl-phosphonium bromide and the appropriate carbonyl compound. To the triphenylphosphonium salt (20 mmol) in anhydrous diethyl ether (100 mL) an equimolar amount of n-butyl-lithium in hexane was slowly added at 0 °C. After 3 h, the appropriate carbonyl compound (20 mmol) was added, the reaction mixture was stirred for 16–20 h at room temperature then, finally, treated with water. The ethereal extract yielded the crude alkene, which was purified by column chromatography on silica gel, using light petrol as eluent in most cases.

3 ^{1}H NMR: 6.50–6.80 (m, 3H, Ar); 5.25–5.90 (s, 2H, OCH_2O); 5.75 (m, 1H, CH=); 4.95 (m, 2H, CH_2=); 2.20–2.65 (m, 3H, $ArCH_2CH$); 0.98 (d, 3H, CH_3).

7 ^{1}H NMR: 0.84 (d, 3H, CH_3-6); 1.17 (s, 6H, $2 \times CH_3$); 1.05–1.55 (m, 7H, H_3, H_4, H_5, H_6), 1.85 (m, 1H, H_7); 2.03 (m, 1H, H_7); 4.93 (bd, 2H, H_9); 5.71 (m, 1H, H_8).

10 ^{1}H NMR: 0.87 (m, 12H, $3 \times CH_3$-4); 1.00–1.35 (m, 6H, H_5, H_6, H_7); 1.5 (m, 1H, H_4); 1.87 (m, 1H, H_3); 2.05 (m, 1H, H_3); 4.97 (d, 1H, H_1); 4.98 (d, 1H, H_1); 5.77 (m, 1H, H_2).

2.4.3. Synthesis of 2,6-dimethylnonan-2-ol (*8*)

Compound **7** (1.0 g; 5.8 mmol) was hydrogenated on Raney nickel to obtain compound **8** in quantitative yields.

^{1}H NMR: 0.96 (t, 3H, CH_3); 1.06 (d, 3H, CH_3); 1.26 (s, 6H, 2 xCH_3); 1.25–1.40 (m, 10H, H_2, H_4, H_5, H_7, H_8); 1.65 (m, 1H, H_6).

Compound **9,** 3,7,7-trimethyloctanal, was synthesised according to Obara et al. (1995). The series of reactions is illustrated in Scheme 1.

2.4.4. Synthesis of 2,2-dimethylpropylmagnesium bromide (*11*)

2,2-Dimethylpropane bromide (0.082 mmol) was slowly added to Mg (2.0 g; 0.082 mol) in anhydrous THF (10.32 mL) under stirring. A small crystal of I_2 was added at the beginning to start the reaction. At the end of the addition, the mixture was refluxed for 1 h.

2.4.5. Synthesis of 6,6-dimethyl-2-hepten-4-ol (*13*)

Crotonaldehyde (**12**) (6.8 mL; 0.082 mmol) in dry THF (10 mL) was added at 0 °C to the Grignard reagent **11**. After refluxing for 2 h, the mixture was hydrolyzed with water and extracted with