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Mini-review

A computational microscope focused on the sense of smell

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"He knew that the senses, no less than the soul, have their spiritual mysteries to reveal. And so he would now study perfumes", Oscar Wilde, The picture of Dorian Gray

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ABSTRACT

In this article, we review studies of the protagonists of the perception of smell focusing on Odorant-Binding Proteins and Olfactory Receptors. We notably put forward studies performed by means of molecular modeling, generally combined with experimental data. Those works clearly emphasize that computational approaches are now a force to reckon with. In the future, they will certainly be more and more used, notably in the framework of a computational microscope meant to observe how the laws of physics govern the biomolecular systems originating our sense of smell.

1. Introduction

We perceive exogenous odorant chemicals through an extraordinary subtle and sensitive system. The sense of smell belongs to the five commonly admitted senses, already cited by Plato in *Theaetetus*, IV centuries BCE. This sense endows us with the perception of chemicals present in our environment and with identification of their intensity with a very high discriminating power [1]. With the sense of taste, it is however considered as a minor sense with respect to senses of earring, seeing and touching. As a matter of fact, quite few people know of how to define an individual lacking the perception of smell (anosmic) or taste (ageusic) but everybody knows what is deafness or blindness. Our brain has nonetheless developed a very powerful mechanism for detecting odorants. At the cellular level, the perception of smell is triggered by activation of our Olfactory Receptor Neurons (ORN). As for the molecular level, volatile odorants driven by the inspired air cross our nasal cavity and reach our olfactory mucosa, itself protected by the olfactory mucus that prevents it from drying out (Fig. 1).

This aqueous layer constitutes a physico-chemical barrier for odorant molecules. Indeed the highly hydrophobic character of odorant molecules hampers them from accumulating within this aqueous phase. Within this mucus, Odorant Binding Proteins (OBP) are the first biological protagonists met by odorants. Despite the fact that their involvement in odor perception is not clearly established, they are thought to bind odorant molecules and transport them to the ORN cilia. At the surface of these ORN cilia, Olfactory Receptors (OR) constitute the cornerstone of the perception of smell. The activation of an OR by an odorant triggers ORN membrane depolarization. These ORNs electric signals spread in our brain and constitute the information we call an odor.

The perception of smell proceeds through a combinatorial code, in which the relation between the odorant space and the receptor space is not a bijection i.e. some odorants can activate several receptors while a single receptor can recognize many diverse odorants [2].

The virtually infinite number of odorant molecules makes it impossible to experimentally screen their potency with respect to the biomolecular protagonists of smell. As a consequence, computational approaches will be of great help to decipher the role of these proteins. They are sufficiently robust to be considered as a computational microscope [3]. In this mini-review, we propose a survey of the knowledge gained on those proteins using molecular modeling. The first paragraph reports on studies dealing with OBP while the second covers studies of OR.

2. Odorant Binding Proteins

OBP are small soluble proteins belonging to the family of lipocalin [4]. They are secreted in the nasal mucus at the quite high concentration of ~10 mM. The exact function of these proteins is not clearly elucidated. The lipocalin family is notably made up of

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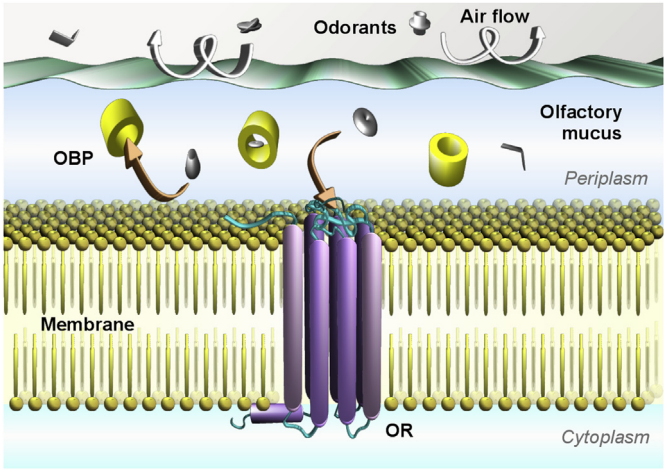

Fig. 1. Schematic representation of the molecular protagonists involved in the perception of smell at the Olfactory Receptor Neuron surface. Odorants molecules (gray) cross the air flow to reach the olfactory mucus (blue). Odorant Binding Proteins (yellow) help solubilize them. Odorants then bind the trans-membrane Olfactory Receptors (purple) to eventually trigger their activation.

transport proteins, such as Retinol Binding Protein (RPB), β-lactoglobulin or Bilin Binding proteins (BBP) for example. These proteins share the property of binding hydrophobic ligands to transport them to an *ad hoc* receptor. By similarity, this suggests that OBP help solubilizing odorants within the mucus to finally activate the receptor, as it has been clearly evidenced in the fruit fly for example [5].

OBP are found in a variety of species including cow, pig, rabbit, mouse, rat, elephant and of course human [68]. Different OBP subtypes have been observed in the same species. For example the mouse has four OBP sub-types, the rabbit three and at least eight can be found in the porcupine. In the rat, three OBP have been described with quite different sequences and binding properties [9–11]. OBP molecular weight is between 17 and 22 kDa. Most OBP are observed as monomers, such as porcine OBP-1, rat OBP-1, rat OBP-3 or human OBP, while some others are found as dimers, such as for the special case of bovine OBP. OBP heterodimers have also been observed in mouse [12].

2.1. Binding properties

OBP reversibly bind odorants with affinities in the micromolar range [7]. As discussed earlier, the fact that a few subtypes are present in most species suggests that OBP do not show a high specificity for a given chemical family. Porcine OBP have been however shown to be post-translationally modified by phosphorylation, generating a diversity of OBP isoforms with specific binding properties [13].

Although, no preferential binding was put forward for the native porcine and bovine OBP. Studies of the three rat OBP revealed intriguing odorant specificities that fulfill each other to encompass all chemical families. Rat OBP-1 preferentially binds heterocyclic compounds such as pyrazine derivatives while OBP-2 appears to be more specific for long-chain aliphatic aldehydes and carboxylic acids. Rat OBP-3 was described as associated to odorants composed of saturated or unsaturated ring structure [9,14–16]. Human OBP-IIA appears unspecific at first sight, as it can associate to various odorant types with dissociation constants in the micromolar range [6]. A chemical specificity for aldehydes and acids, either aliphatic or aromatic was however revealed [17].

2.2. Sequence & structure

All lipocalins have generally a low percentage of identity (~25%). The maximal known identity of 42% is found between a monomer of the bovine and the porcine OBP and the lowest concern rat OBP-2 (12–19%). The hallmarks of their sequence are scarce: a GxW motif is found ~15–20 residues away from the N-terminus, two cysteine residues in the middle of the sequence and a glycine residue at the C-terminal end.

From a structural point of view, OBP share the typical lipocalin fold. They are composed of an eight-strand β-barrel, flanked by seven loops. An α-helix followed by a small β-sheet is present at the C-terminal end, as shown in Fig. 2. A cysteine residue on the D strand of the β-barrel is engaged in a disulfide bridge with the C-terminal domain.

The β-barrel constitutes a *calyx* bearing a *lipophilic* cavity. This property is at the origin of their family name (lipo/calyx). The bovine OBP is a bit special. It is made up of an oligomer, where two lipocalin structures have underwent a so-called 'domain swapping' through an exchange of their α-helices [18].

2.3. Molecular modeling of OBP

The rather easy way to express those hydrosoluble proteins has allowed solving crystal structures for many of them. It follows that at the exception of a few cases, molecular modeling studies are most of the time based-on an experimental structure. In many research articles, molecular modeling was mainly used with the purpose of identifying the protein binding site. It's only more recently that the function of these proteins was investigated by means of theoretical approaches.

Through a series of explicit solvent molecular dynamics studies, the dynamic behavior of OBP has been described. The volume of the binding cavity was computed to ~500 $Å^3$ and showed fluctuations between 400 and 800 $Å^3$ during the MD simulations. Although occluded from the bulk water, the binding cavity showed transient openings, notably at the junction between loop1 and strands D and E of the β-barrel [19]. Unconstrained [20] or constrained [21] molecular dynamics simulations observed the binding or unbinding of odorants through the opening of this part of the protein, confirming that it is the main access from the bulk to the binding cavity. Gratifyingly, the crucial role of a highly conserved tyrosine residue (Y82) initially pointed out in the simulations was experimentally confirmed afterward [13].

Once bound to the OBP, the odorant is engaged in an opportunistic interaction with the protein involving a few hydrogen-bonds [22]. If hydrogen bonds are observed, they possess short residence time since the position of the odorant within the cavity is subjected to hydrophobic contacts. The number of these contact together with the size of the odorant appear to be the main factor originating high affinity [16,19]. More elaborated protocols allowed computing the affinities between these unspecific proteins and series of odorants. State-of-the-art free energies were able to predict with a high accuracy the differential affinity between closely related ligands, showing that a computational deorphanization of OBP is possible [23]. More modest models, solely based on ligands structures, such as pharmacophore approaches, were proved unable to predict the affinity while docking and end-point MM-GBSA [24] free energy approaches perform equally at reproducing an experimental ranking between odorants with respect to their affinity [25].

Focusing on human OBP, Tcatchoff et al. proposed a model to investigate the intriguing selectivity of hOBP-IIA for aldehydes and acids [17]. This protein has no crystal structure available and a homology-based model was built using the human tear lipocalin as a template. Although the binding mode is not associated to strong