The Debate over Rational Design of Odorants

The hypothesis of olfactory receptors as metalloproteins and the future of odorant design

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In his recent volume, *The Secret of Scent: Adventures in Perfume and the Science of Smell*, Luca Turin describes his passion for perfume and his pursuit of olfactive mysteries.[1] Turin re-broached the vibrational theory of olfaction and proposed a transduction mechanism of primary olfactory reception. The scientific community has been skeptical of this mechanism, which relies on the assumption that olfactory receptors are metalloproteins, a hypothesis that has not received much attention but which deserves further investigation.

Background

Turin began collecting perfumes as a hobby in the 1980s. His ability to precisely describe scents enabled him to write his renowned *Parfums: Le Guide* (1992), which became the best-selling perfume guide in France and granted him access to the secretive big fragrance corporations.[2] With this insight into the perfume business, he learned that the creation of a new odorous molecule is a tedious and costly endeavor, highly reliant on experimental trial and error. Driven by an impulsive curiosity, Turin decided to devote his research activity to unraveling the fundamentals of olfaction, resulting in a controversial theory. Details about how this theory was devised and the problems he faced in putting it forward to the scientific community are outlined in Chandler Burr's well-known 2003 book *The Emperor of Scent*.[3]

In *The Secret of Scent*, Turin outlines the steps involved in the creation of new perfumes, illustrating the technology, science and art behind every fragrance found on the market. Next, he outlines fragrance chemistry and describes the complex relationships between molecular structure and odor for the most relevant odor classes in perfumery. Finally, he provides a detailed look at the relevant theories of olfaction that in recent decades have attempted to interpret odor character.

Origins of Debate

In the 1930s, Dyson observed that in some cases odor character was related to the presence of certain functional groups, and not so much with molecular shape.[4] Given that each functional group is characterized by particular patterns in the vibrational spectra, he speculated that olfactory receptors (ORs) were able to probe the molecular vibration of a bound odorant, a hypothesis further extended by Wright.[5] But no transduction mechanism was proposed to interpret how ORs could detect vibrations, and so the scientific community was reticent to accept the idea of ORs working as biological spectroscopes. Amoore popularized the idea that molecular shape is related to odor character.[6] His stereochemical theory became widely accepted, particularly following the discovery of the large family of genes encoding ORs.[7] A more modern approach suggests that ORs probe the shape not of the whole odorant but of partial molecular features, referred to as odotopes. Although the underlying basis of the stereochemical theory still persists, it has been renamed *weak-shape*, or *odotope*, theory.[8] Yet this theory can hardly interpret the many irregularities of olfaction that Turin collected from searching the literature and other sources, as described in his book.

An Alternative Model

Based on his professional experience in biophysics—from 1993 to 2000 he was a lecturer on the subject at University College London—Turin finally assembled various pieces of evidence into a complex puzzle, which led to a peculiar transduction mechanism of primary olfactory reception.[9] This theory was based on inelastic electron tunneling spectroscopy, which relies on the interactions between electrons tunneling across a narrow gap between metallic electrodes.[10] Metallic conductors are absent in biology, but Turin suggested that electron tunneling was possible with proteins containing a metal ion able to interact with the odorant. He intimated that a zinc ion was the one likely to be involved in odorant recognition. A recent work has further studied the physical viability of this mechanism, and the results were consistent with observed features of smell.[11]

In 2001, Turin joined Flexitral, a private enterprise that uses rational design to develop fragrance molecules. The company has been rather successful in the development of novel odorants by computational methods derived from Turin's theory.[12] He claims that the success rate was one product in 10 molecules synthesized, two orders of magnitude better than the industry standard of one in 1,000.[1,13]

The scientific community has been reluctant to accept Turin's theory because no similar mechanism has ever been described in a biological system. However, it is based on the assumption that olfactory receptors are metalloproteins, a hypothesis consistent with the reported evidence of a metal binding motif conserved in ORs.[14] The assumption that odorant-receptor interactions might be mediated by a metal ion in some receptors has yet been neither validated nor disproved, but it allows the interpretation of different properties of olfaction and is reasonable from an evolutionary standpoint, as described below.

The Effect of Functional Group in Odor Character and Intensity

Chemists noticed long ago that the presence of certain chemical groups in a molecule is frequently correlated with a particular odor that can be detected by trained observers, especially in small molecules. So, the thiol moiety (-SH) imparts to any molecule, regardless of its shape, a unique sulfuraceous odor character related with the smell of rotten eggs or garlic. When nitriles (-CN) are used as chemically stable replacements for aldehydes, they impart an oily-metallic character to any odorant; isonitriles (-NC) produce a flat metallic character of great power and unpleasantness; oximes (-NOH) give a green-camphoraceous character; nitro groups ($-NO_2$) produce a sweet-ethereal odor; isothiocyanate groups (-NS) result in a mustardy smell; amine groups ($-NH_2$) produce a fishy-urinelike odor in any molecule; arsine groups ($-AsH_2$) smell like cabbage; and esters [-(C=O)-O-] usually smell fruity.[3]

Based on the observed correlation between functional groups and odor character, Beets proposed the so-called *profile-functional group* theory.[15] He suggested that the smell of a given odorant is determined by two separate contributions: one from the form, size and bulk shape of the molecule, and the other from its functional group, which determines the orientation of the odorant at the receptor site.

One interesting pair of structurally related compounds with greatly different odors is methanol, which is relatively odorless, and methyl mercaptan, which has a highly powerful and disagreeable odor. Neither the slight differences in bond length, bond angle or reactivity, nor the slight difference in orientation of the methyl groups, appear capable of explaining such pronounced difference in odor quality and intensity. Klopping pointed out that the major difference is the ability of mercaptans to form stable complexes with many metal ions.[16]

Acetonitrile (CH_3-C≡N:) presents a molecular structure similar to methyl isonitrile (CH_3-N^+≡C^-:). Both have large dipole moments with a lone electron pair on the terminal atom of the functional group that can play the role of hydrogen bond acceptor. Moreover, both are linear molecules of nearly the same size and shape. However, acetonitrile has a relatively weak, pleasant, ethereal odor, while methyl isonitrile has an extraordinarily vile and powerful odor. According to Klopping, isonitriles, unlike nitriles, react with salts of many heavy metals to form very stable complexes, which would account for the remarkable difference in odor quality.[16]

Chemists observed long ago that those odorants that are good ligands for metal ion coordination are likely to possess high odor intensity.[14] Turin pointed out that thiols, amines, nitriles and isonitriles, some of which are among the strongest odorants known, coordinate with zinc readily. Other strong odorants like emoxyfurone, oxathiane, vanillin, diacetyl and pyrazine esters present structural features capable of bidentate binding to a metal ligand.[9] Ohloff compared the molecular structure of strong-weak stereoisomer pairs of odorants and suggested that when two hydrogen bond acceptors are present, the odorant smells stronger when they are close to each other.[17] This observation, known as bifunctional rule, can be interpreted in some cases, assuming that the strong isomer is a bidentate ligand for zinc, whereas the weak isomer has unfavorable geometry for zinc-binding.[8] Although this rule is particularly

interesting because it applies to a large number of structurally unrelated odorants, there are many exceptions, and steric restrictions probably also play a key role in odor potency because certain molecular shapes are more favorable than others in binding the target receptors.

These examples suggest that the recognition mechanism must somehow be sensitive to the fine structure of the electron distribution—orbital energies, charge density, etc.—of the functional group.[8] This aspect is not properly taken into account by the odotope theory, but is consistent with the assumption of a metal ion involved in odorant recognition (at least in some ORs). This hypothesis was used by Turin to devise his theory, and it would explain why odor intensity spans over several orders of magnitude for compounds with similar size and volatility but different functional groups or molecular features.[9]

The Metal Ion-assisted Odorant Recognition Mechanism

Another mechanism proposed in 2003 also assumed that ORs are metalloproteins. Searching the genome sequences of human ORs, Wang et al. found that the consensus sequence HxxC[DE] (histidine; two residues usually hydrophobic; cysteine; aspartic or glutamic acid) was rather conserved in the 4-5 loop, i.e., the extracellular loop between the fourth and fifth transmembrane (TM) α-helices.[14] Interestingly, the 4-5 loop is of functional importance in ligand binding for other G-protein coupled receptors (GPCRs) like the cholecystokinin-B receptor and aminergic receptors.[18,19] In rhodopsin, two residues of this loop were reported to interact with retinal.[20]

To test the metal-binding properties of this sequence, a pentapeptide containing this putative binding site was synthesized, and it was discovered that this motif had a high affinity to bind different metal ions like Cu(II), Zn(II) and Ni(II). So, it was suggested that those odorants with a high affinity for metal ion coordination will bind most tightly to a specific subset of ORs, resulting in a strong odor perception.[14] Among these metal ions, Zn(II) is probably the best candidate for several reasons: it strongly coordinates amines and thiols, which are strong odorants; it is widely distributed throughout the central nervous system; and zinc deficiency is unique in causing a complete and rapidly reversible anosmia.[9,14,21] Moreover, Zn(II) binds with high affinity to and modulates the function of a number of seven-transmembrane proteins in neural tissues such as the tachykinin NK3 receptor and the β_2-adrenergic receptor.[22,23]

The tertiary structure and activation pattern of ORs is usually assumed to be similar to rhodopsin. Different studies have revealed that photoactivation of rhodopsin involves a rotation and tilting of TM 6 relative to TM 3.[24] On the contrary, Wang et al. proposed a dramatic conformational change of the receptor upon odorant binding that involved membrane penetration of the 4-5 loop and replacement of one TM α-helix.[14] No similar mechanism has been described for any other GPCR, and probably for this reason it has not received much attention yet. However, this mechanism seems more plausible than Turin's theory, and other alternative conformational arrangements should be considered for further investigation.

Odorant Recognition Details in mOR-EG

Details about odorant-receptor interaction at the molecular level have been recently reported for the mouse receptor mOR-EG. Katada et al. conducted a computational structural model for this receptor and identified 10 amino acids supposedly involved in the odorant binding site.[25] Site-directed mutagenesis of these amino acids and subsequent odorant binding assays confirmed their role in odorant recognition. Although the mechanisms of OR activation upon ligand binding are still uncertain, these results provide a strong functional evidence for the odotope theory, but no metal ion was assumed to be involved. However, mOR-EG contains the motif HFFCE in the 4-5 loop, which might strongly bind a metal ion. Site-directed mutagenesis of this motif would provide valuable information regarding the role of the 4-5 loop in ORs. Further studies will be necessary to investigate if similar patterns of odorant recognition apply for all ORs. In any case, based on the evidence stated above, I speculate that some ORs contain a metal ion directly involved in odorant recognition.

Evolutionary Interpretation of the Role of Metal Ions in Olfaction

Throughout evolution, olfactory receptors essentially specialized in the identification of food and potentially harmful conditions and factors, such as predators, putrid food, fire and toxic gases. Amines and thiols are associated to the latter, and maybe for this reason they smell unpleasant. So, putrid fish produces trimethylamine, while the degradation of meat releases thiols and hydrogen sulfide (H_2S), given that two amino acids contain sulfur. Another harmful condition is the presence of toxic gases. Animal cells need oxygen for their metabolism, which is carried out by hemoglobin. This protein contains 4 heme prosthetic groups, and each one tetrahedrally coordinates a ferrous ion, Fe(II). This ion forms a coordinate bond with one electron pair of oxygen, which allows the transport of this molecule (O_2).

Hydrogen sulfide has a high affinity to form coordinate bonds with metal ions, including Fe(II) in hemoglobin. Thus, H_2S acts as a competitive inhibitor of hemoglobin, interfering in the transport of oxygen and causing grave toxicity. The same mechanism occurs with cyanide (CN^-), sulfur monoxide (SO), nitrogen dioxide (NO_2) and sulfide (S^-). Ammonia has a high affinity for metal coordination as well, but the inhibition of hemoglobin is not the main cause of toxicity; this gas reacts with water, forming ammonium hydroxide, which produces chemical burns in the respiratory system. These toxic gases were present occasionally in the atmosphere in early stages of life's evolution on Earth; for example, volcanoes release sulfurous compounds. Thus, it is reasonable to assume that olfaction evolved in order to detect the presence of these gases. Similarly, other authors have suggested that the high sensitivity of human olfaction in detecting hydrogen sulfide and amines is an evolutionary adaptation for detecting decaying food and toxic gases, which have been present for evolutionarily significant time periods in the atmosphere.[26]

Given that those small molecules able to form a coordinate bond with the ferrous ion of the heme group are toxic because they inhibit oxygen transport, it is reasonable to assume that olfaction adapted to detect them through a similar mechanism, with a metal ion involved in odorant recognition at least in some ORs. This hypothesis presents two remarkable exceptions. Hemoglobin has a binding affinity for carbon monoxide (CO) 200 times greater than its affinity for oxygen, and hence this gas is toxic at low concentrations. However, CO is odorless. Nitric oxide (NO) also inhibits the transport of oxygen and has little or no odor. The reason could be that CO and NO are used by the olfactory system as neurotransmitters, and consequently their odorless character might be due to other reasons.[27]

A recent study has found several types of P-450 cytochromes expressed preferentially in the nasal mucosa.[28] These enzymes rapidly inactivate and extinguish odorants received by a given sniff, which is crucial for a fast detection of predators and other key tasks for survival. Interestingly, P-450 cytochromes contain a heme group. Thus, although their effect in odor character and intensity is still uncertain, it may be inferred that those odorants able to form a coordinate bond with ferrous ions, like thiols or amines, might act as competitive inhibitors of these enzymes, resulting in a higher concentration in the olfactory

epithelium, and hence a stronger odor. So, the stronger smell of these odorants could be due to a high affinity to activate target ORs—assuming a metal ion is involved in the binding site—and a simultaneous, possibly synergic, inhibitory effect of P-450 cytochromes.

The Future of Rational Odorant Design

The research group of H. Matsunami has developed a high-throughout platform for screening the chemical selectivity of the large OR family.[29] It has cloned 300 human and 250 mouse ORs using HEK293-T cells. The activity of these ORs was tested with a group of 78 odorants, and it was found that 20 human and 80 mouse ORs were activated by some of the compounds. This work in progress, still unpublished, was presented at the 28th AChemS conference.[30] A private enterprise is also involved in a similar project (*www.chemcom.be*).

This technology opens new possibilities for the discovery of new odorants with a particular odor character. The first step would be to take a few odorants with the desired character as well as other molecules with a similar structure but a different odor. Screening this set of compounds with all human ORs will allow the identification of selective ORs activated by one or a few of these odorants. Those receptors selectively activated by the odorants of interest and not by the analogs will likely code for the desired odor character. The next step would be to conduct computational structural models for these particular ORs, taking into account similar studies reported in the literature in order to identify the key amino acid residues involved in odorant recognition.[31] These models can be validated by target mutagenesis studies as in the case of mOR-EG described above.[25] Then, docking simulations can be carried out with libraries of virtual molecules, which would lead to the identification of novel odorants with a high affinity to activate the ORs specific to a particular odor character. Although the success of this methodology is not guaranteed, given that the details of OR activation are still uncertain, as well as the factors that affect odor intensity—especially the perireceptor events involved in odorant transport and biotransformation—this technology is likely to become the future of rational odorant design.

Conclusion

Turin emphasized that the odotope theory can hardly interpret many irregularities of olfaction, and he devised a mechanism for primary olfactory reception based on the vibrational theory. Although the idea that ORs can detect molecular vibration is controversial, this mechanism is based on the assumption that ORs are metalloproteins. This hypothesis is still pure conjecture, but it is consistent with different pieces of evidence reported in the literature concerning ORs and other GPCRs. Moreover, it is reasonable from an evolutionary standpoint. Further functional assays are still necessary to derive the mechanisms of odorant recognition and the details of OR activation, which will determine the basis the future technology for rational odorant design.

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