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Abstract

The human olfactory system allows to perceive and identify a huge number of odours from few hundreds ORs involved in an olfactory coding whereby one olfactory receptor (OR) recognises multiple odorants while one odorant activates different combinations of ORs. Odours perceived in our environment are mainly the result of mixtures of odorants, but the specific mechanisms involved in their processing remain poorly understood. In previous studies performed at INRAE-CSGA, the perception of a binary mixture of ethyl isobutyrate (Et iB, strawberry-like odour, STR) and ethyl maltol (Et-M, caramel-like odour, CAR) was investigated in comparison with a reference (allyl hexanoate, Al-H, pineapple-like odour, PNA). In humans, the binary specific mixture of Et-iB and Et-M was judged as more typical of a pineapple odour than the individual components, and similar to those of allyl hexanoate. The analysis of the network of odours sharing by 293 molecules described with at least one of the odours STR, CAR or PNA revealed peculiar links between odours, and led to identify 9 STR-CAR and 4 STR-PNA molecules. We investigated the molecular features of these molecules by performing pharmacophore generations using the STR-CAR, STR-PNA sets, both separately and putting together the 13 molecules. Comparing the distances between features of the three models revealed a common distance close to 8 Å between the centres of at least one HY and one HBA. Additionally, the pharmacophore comparison of the three models showed a satisfactory mapping of the features. These results support the hypothesis wherewith molecules sharing the odours involved in a blending mixture could recognise a common set of ORs.

Keywords: odour notes, aroma blending mixture, pharmacophore

Introduction

The perception of the odours begins at the peripheral olfactory system by the interactions of odorants with olfactory receptors (ORs) in the nose [1]. The perception and discrimination of a huge number of odours from few hundreds ORs involves an olfactory scheme whereby one OR recognises multiple odorants while one odorant activates different combinations of ORs [2]. Nevertheless, in spite of advances in the knowledge of olfactory perception, the pathway(s) involved in the odours perception remains poorly understood [3, 4]. It is especially challenging in the case of mixtures of odorants [5, 6], while odours perceived in our environment essentially stem from mixtures of odorants [7]. In some cases, the olfactory processing of a mixture of odorants produces a homogeneous percept in which a single odour is perceived from the mixture thanks to a configural process [5, 7]. Odour blending occurs if a mixture of molecules A and B carrying different odours is perceived to have a specific new odour AB, distinct from the odours of each component A and B [8]. Thus, a blending mixture percept can be represented as AB ≠ A+B.

Currently, target approaches that concern the interactions of odorants at the OR level are most often undertaken [9-11]. However, in our own study, we focused on a ligand approach that is complementary to the target approach. In the context of aroma blending, we considered a set of odorants, whose selection was based on an aroma blending previously carefully investigated in several studies performed with animals [12, 13] and humans [14-16]. These studies constantly revealed that the perception of a mixture of ethyl isobutyrate (Et-iB), which has a strawberry-like odour (STR), and ethyl maltol (Et-M), which has a caramel-like odour (CAR) is configurally processed by the olfactory system. The binary specific mixture of Et-iB + Et-M was investigated in humans in comparison with allyl hexanoate (Al-H), which has a pineapple-like odour (PNA) and was chosen as reference to evoke an odour close to the one expected in the mixture. It was established that the mixture has an odour close to this reference. Moreover, the binary mixture was judged as having an odour more typical of pineapple than of the individual components [14].

To explore the key features of this aroma blend we built a dataset of 293 molecules by selecting in a large flavour database [17, 18] the odorants having at least one of the odours STR, CAR or PNA. In a recent study [19], we have analysed through a network the co-occurrences of the odour notes in the descriptions of the odorants. The

DOI: 10.5281/zenodo.5948755

odours network revealed peculiar links between odours, especially this analysis led to identify 9 STR-CAR and 4 STR-PNA molecules.

Recognising that molecules sharing analogous odour qualities could possess common structural molecular properties, and that combinations of activated ORs encode odour qualities [20, 21], we hypothesised that molecules sharing the common STR odour note should have some common structural features.

With the aim to investigate the structural features of these molecules, we developed an *in silico* approach using pharmacophores study. The pharmacophore generations [22] were performed using the STR-CAR, STR-PNA and STR-CAR+STR-PNA subsets. Comparing the inter-features distances and testing the mapping of the pharmacophore models allowed to put forwards the common as well as the peculiar characteristics of each subset.

Experimental

Data Preparation

The molecules STR-CAR and STR-PNA were extracted on the basis of their odour notes from the large database [18] designed from the 9th version of Flavor-Base [17]. The three subsets STR-CAR, STR-PNA and STR-CAR+STR-PNA encompass respectively 9, 4 and 13 odorants.

Computational Chemistry

The computational analyses were conducted using Discovery Studio 2021, BIOVIA [23] running on Windows 10 for PC. The pharmacophores were generated using the HipHop/Catalyst protocol implemented as the "Common Feature Pharmacophore Generation" protocol in Discovery Studio 2021[22]. A maximum of 250 conformers were generated in a range of 0-20 kcal/mol [24]. The maximum number of generated hypotheses for each run was set to 10. We considered the following pharmacophoric features: hydrogen bond acceptors (HBA features), lipid hydrogen bond acceptors (HBA-lip features), hydrophobic regions (Hy features) and hydrophobic aliphatic regions (Hy-al features). Because the size of the odorants (74<MW<260), the parameter "Minimum Interfeature Distance" was decreased from to 0.5 Å. The minimum number of feature points were set to 2. All the molecules were regarded as "Active". The maximum omitted features parameter ("MaxOmitFeat") that specifies how many features the generated pharmacophore is allowed to miss for each molecule was set to 0 for all molecules (means that all features must map to this molecule).

The pharmacophores were compared using the "Pharmacophore Comparison" protocol, which allows the mapping and alignment of two pharmacophores; an RMSD value is reported for the matching pharmacophore features. The "Best Mapping Only" parameter was used for the comparisons. The terms "pharmacophore [model]" and "hypothesis" refer interchangeably to the assemblage of features required for the biological activity of the ligands oriented in 3D space [22].

Results and discussion

Most of the subsets are characterised by a specific number of acyclic or cyclic structures. The STR-PNA molecules are acyclic esters with saturated, branched and/or unsaturated chains of 7 or 8 carbons, except for ethyl cis-4-decenote ($C_{12}H_22O_2$), which is larger than the other compounds. Conversely, all the STR-CAR molecules except one (2-Methyl-2-pentenoic-acid) have monocyclic structures derived from maltol or furan. It should be noted that four molecules in the STR-CAR subset are artificial maltol derivatives. Thus, the majority of STR-CAR odorants are furan derivatives. The list of STR-CAR and STR-PNA odorants is reported in Table 1.

Table 1. List of the 13 odorants STR-CAR and STR-PNA.

Odorant name	cas_no	nature	subset odor
Dimethylethoxyfuranone	65330-49-6	Nature identical	STR-CAR
Furaneol butyrate	114099-96-6	Nature identical	STR-CAR
Hydroxymethylfuranone	19322-27-1	Nature Identical	STR-CAR
Maltol	118-71-8	Nature Identical	STR-CAR
2-Methyl-2-pentenoic acid	3142-72-1	Nature Identical	STR-CAR
Ethyl maltol isobutyrate	852997-28-5	Artificial	STR-CAR
Ethyl maltol propionate		Artificial	STR-CAR
Maltol Propionate	68555-63-5	Artificial	STR-CAR
Maltyl 2-methylpropanoate	65416-14-0	Artificial	STR-CAR
Ethyl cis-4-decenoate	7367-84-2	Nature Identical	STR-PNA
Ethyl hexanoate	123-66-0	Nature Identical	STR-PNA
Isopropyl butyrate	638-11-9	Nature Identical	STR-PNA
Ethyl 2-methyl-3-pentenoate	1617-23-8	Artificial	STR-PNA

To examine the critical common features present in these subsets of odorants, we performed a pharmacophore approach using the HipHop/Catalyst protocol implemented in Biovia Discovery Studio [23]. Our study was carried out on the following training sets: STR-CAR (9 odorants), STR-PNA (4 odorants) and STR-CAR+STR-PNA (13 odorants).

All the models generated from the three subsets are made up of 2 HBA-lip features. However, STR-PNA model is the only one that has two Hydrophobic feature (HY) while there is only one for STR-CAR and STR-CAR+STR-PNA models.

The inter-features distances and the alignments of odorants of each subset on the corresponding generated pharmacophore and are displayed in Figure 1.

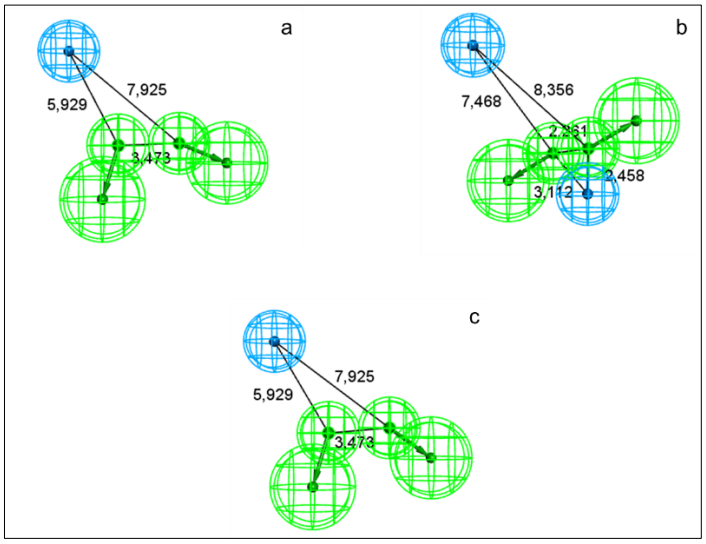

Figure 1*: Inter-features distances in Å for the pharmacophores Hypos_01 generated from the corresponding subsets: (a) STR-CAR; (b) STR-PNA; (c) STR-CAR+STR-PNA.*

The distances between the features of Hypo_01 generated from the STR-CAR and STR-CAR+STR-PNA are the same, and the two models are identical (RMSD=0).

Comparing the distances between features of the two models generated by STR-CAR and STR-PNA revealed a common distance close to 8 Å between the centres of at least one HY and one HBA. The pharmacophore comparison (Figure 2) reveals a satisfactory mapping of the two models (RMSD= 1.189394 Å).

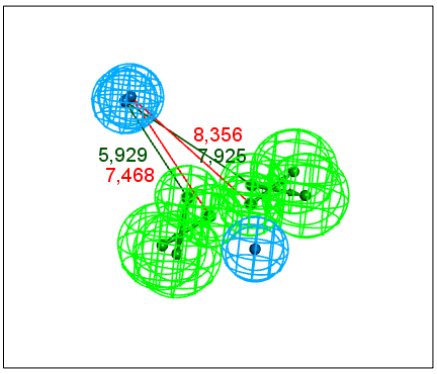

Figure 2*: Pharmacophore mapping by pairs of the two pharmacophores models Hypos_01: STR-CAR and Hypo1_STR-PNA. The distances between Hypo1_STR-CAR features are shown in dark green and the distances between Hypo1_STR-PNA features are shown in red.*

Conclusion

Despite the diversity of molecular structures of molecules STR-CAR and STR-PNA, the generated pharmacophores possess some common characteristics, especially a common distance close to 8 Å between the centres of at least one HY and one HBA, which allows a satisfactory overlap among the models.

These obtained results agree with the scheme of olfactory coding and with the assumption whereby molecules sharing the odours sharing a common odour note could recognise a common pattern of ORs.

This research was funded by Agence Nationale de la Recherche, ANR-18-CE21-0006 MULTIMIX, and INRAE-TRANSFORM department (ANS CAMELIA).

References

1. Buck L, Axel R. A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition. Cell. 1991;65(1):175-87.
2. Malnic B, Hirono J, Sato T, Buck LB. Combinatorial receptor codes for odors. Cell. 1999;96(5):713-23.
3. Block E. Molecular Basis of Mammalian Odor Discrimination: A Status Report. J Agric Food Chem. 2018;66(51):13346-66.
4. Genva M, Kemene TK, Deleu M, Lins L, Fauconnier ML. Is It Possible to Predict the Odor of a Molecule on the Basis of its Structure? Int J Mol Sci. 2019;20(12):3018.
5. Berglund B, Berglund U, Lindvall T. Psychological processing of odor mixtures. Psychol Rev. 1976;83(6): 432-41.
6. Singh V, Murphy NR, Balasubramanian V, Mainland JD. Competitive binding predicts nonlinear responses of olfactory receptors to complex mixtures. Proc Natl Acad Sci U S A. 2019;116(19):9598-603.
7. Thomas-Danguin T, Sinding C, Romagny S, El Mountassir F, Atanasova B, Le Berre E, et al. The perception of odor objects in everyday life: a review on the processing of odor mixtures. Front Psychol. 2014;5:504.
8. Wiltrout C, Dogra S, Linster C. Configurational and nonconfigurational interactions between odorants in binary mixtures. Behav Neurosci. 2003;117(2):236-45.
9. Pfister P, Smith BC, Evans BJ, Brann JH, Trimmer C, Sheikh M, et al. Odorant Receptor Inhibition Is Fundamental to Odor Encoding. Curr Biol. 2020;30(13):2574–87.
10. Xu L, Li WZ, Voleti V, Zou DJ, Hillman EMC, Firestein S. Widespread receptor-driven modulation in peripheral olfactory coding. Science. 2020;368(6487):eaaz5390.
11. McClintock TS, Wang Q, Sengoku T, Titlow WB, Breheny P. Mixture and Concentration Effects on Odorant Receptor Response Patterns In Vivo. Chem Senses. 2020;45(6):429-38.
12. Coureaud G, Thomas-Danguin T, Le Berre E, Schaal B. Perception of odor blending mixtures in the newborn rabbit. Physiol Behav. 2008;95(1-2):194-9.
13. Coureaud G, Thomas-Danguin T, Wilson DA, Ferreira G. Neonatal representation of odour objects: distinct memories of the whole and its parts. Proc R Soc B-Biol Sci. 2014;281(1789):20133319.
14. Le Berre E, Thomas-Danguin T, Beno N, Coureaud G, Etievant P, Prescott J. Perceptual processing strategy and exposure influence the perception of odor mixtures. Chem Senses. 2008;33(2):193-9.
15. Barkat S, Le Berre E, Coureaud G, Sicard G, Thomas-Danguin T. Perceptual Blending in Odor Mixtures Depends on the Nature of Odorants and Human Olfactory Expertise. Chem Senses. 2012;37(2):159-66.
16. Sinding C, Coureaud G, Bervialle B, Martin C, Schaal B, Thomas-Danguin T. Experience shapes our odor perception but depends on the initial perceptual processing of the stimulus. Atten Percept Psychophys. 2015;77(5):1794-806.
17. Leffingwell. Flavor-Base 9th Ed. 2013.
18. Tromelin A, Chabanet C, Audouze K, Koensgen F, Guichard E. Multivariate statistical analysis of a large odorants database aimed at revealing similarities and links between odorants and odors. Flavour Frag J. 2018;33(1):106-26.
19. Tromelin A, Koensgen F, Audouze K, Guichard E, Thomas-Danguin T. Exploring the Characteristics of an Aroma-Blending Mixture by Investigating the Network of Shared Odors and the Molecular Features of Their Related Odorants. Molecules. 2020;25(13):3032.
20. Furudono Y, Sone Y, Takizawa K, Hirono J, Sato T. Relationship between Peripheral Receptor Code and Perceived Odor Quality. Chem Senses. 2009;34(2):151-8.
21. Saito H, Chi Q, Zhuang H, Matsunami H, Mainland JD. Odor coding by a Mammalian receptor repertoire. Sci Signal. 2009;2(60):1-14.
22. Clement OO, Mehl A. HipHop: Pharmacophore Based on Multiple Common-Feature Alignments. In: Güner OF, editor. Pharmacophore perception, Development and Use in Drug Design. IUL Biotechnlogy Series. La Jolla: International University Line; 2000. p. 69-84.
23. Discovery Studio 2021, Dassssault Systèmes Biovia Corp.
24. Smellie A, Teig SL, Tobwin P. Poling: Promoting Conformational Variation. J Comput Chem. 1995;16(2):171-87.