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16th ECRO Biennial Congress, Dijon, 12-15 september 2004

CONFORMATIONAL CHANGE OF THE RAT ODORANT-BINDING PRO... F UPON ODORANT BINDING

RA

C Nespoulous, L Briand, M-M Delage, V Tran and J-C Perno...

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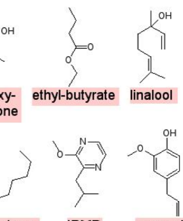
...troduction

...ctory binding proteins (**OBPs**) are small abundant ...lular proteins which participate in peri-receptor events of ...and vertebrate olfaction. Their are thought to transport ...e hydrophobic odorant molecules through the sensillum ...or the aqueous nasal mucus, respectively, in order to reach ...embrane receptors embedded in the membrane of olfactory ...s.

...Ps belong to the **lipocalin superfamily**. Although members ...family display usually less than 20% sequence identity, all ... conserved folding pattern, an 8-stranded β-barrel flanked ...-helix at the C-terminal end of the polypeptide chain. The β-...defines a **central apolar cavity, called calix**, whose role is ...and transport hydrophobic odorant molecules.

...Ps have been identified in a variety of species. When ...t subtypes occured in a same animal species, OBPs were ...to be specially tuned towards distinct chemical classes of ...s.

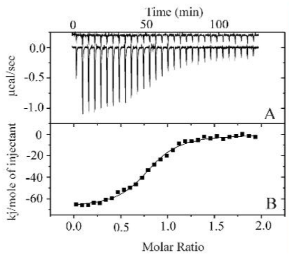
...report the **binding properties** for several odorants and ...**ral conformational change** upon ligand binding of the **rat OBP-1F**, heterogously secreted using the yeast *Pichia* ...t.



...al structure of the odorants ...n the binding experiments

In addition to IBMP, a model odorant systematically used in OBP studies, the tested odorants were chosen among molecules largely used in the food industry, exhibiting different odors and persistence and belonging to different chemical classes.

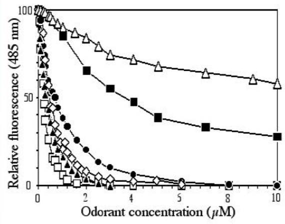
Determination of thermodynamic parameters



Calorimetric titration of the binding of Eugenol to OBP-1F at 30°C, pH 7. (A) Raw data of ITC experiment. (B) Binding enthalpy corrected for heat of ligand dilution (upper curve fig A).

Eugenol binding was saturable with about **one apparent site per dimer**, as indicated by the abscissa of the inflection point.

1-AMA fluorescence competitive binding curves. hydroxy-butanone (Δ), linalool (●), ethyl-butyrate (■), IBMP (▲), γ-decalactone(◇), eugenol (□)



Titration of OBP-1F with 1-AMA was saturable (Kd=0.6μM) with **one binding site per dimer**. Tested odorants displaced with different ability the fluorescent probe 1-AMA. The measured half-maximal values (IC_{50}) allowed to calculate an **apparent dissociation constants (K_{diss}) in the micromolar range** for the 4 most active odorants, and an order of magnitude smaller for ethyl-butyrate and hydroxy-butanone.

The **discrepancy** in the magnitude of values **between both measurements** was likely due to the complexity of the indirect measurement of the fluorescence assay. The value of IC_{50} provide only apparent constants, only useful to classify ligands tested in the same experimental conditions.

Bindi...s

Thermodynamic paramet... OBP-1F obtained by ITC and ...ined ... fluorescence competitive ...scence measurements were carried ...er at neu... Dashes indicate impossibilit...

Odorants	ΔH kJ/mole	ΔS J/...	[illegible]	[illegible]	[illegible]	(a)1/K... 10...
Hydroxy-butanone	-	[illegible]	[illegible]	[illegible]	[illegible]	0.09
Ethyl-butyrate	-34.1 ± 1.4	-2...	[illegible]	[illegible]	...11	0.29
γ-decalactone	-64.8 ± 0.7	-98.0	-35.1	0.84 ± 0.01	1.10 ± 0.08	9.63
Linalool	-42.7 ± 0.8	-19.6	-36.7	0.61 ± 0.01	2.14 ± 0.33	6.67
IBMP	-35.3 ± 0.9	4.2	-36.6	1.15 ± 0.02	2.32 ± 0.57	14.4
Eugenol	-63.1 ± 1.1	-86.4	-36.9	0.68 ± 0.01	2.41 ± 0.33	21.0

(a) K_a^{ITC}: association constant determined by ITC experiments;
(b) K_{diss}^{fluo}: apparent dissociation constant determined by fluorescent experiments.

The **affinity constants** of **OBP-1F** were found to be ... **micromolar range**, as observed for other vertebrate OBP.

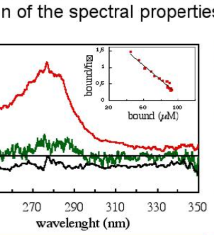
The **binding stoichiometry** for the odorant-**OBP-1F** inte... did not reach **one site per dimer**, except for IBMP, as obse... many cases. It can be explained by the presence of ... inactive proteins or by the occupation of binding sites by li... ligands tightly bound onto OBP, not removed durin... purification process.

Odorant affinities should be correlated to their molar **m... length or size** which appeared to be **a major selective c... for recognition** among the structural features of odorants i... in response of olfactory system observed at different levels.

The Gibbs free energy of each interaction was simil... differences of ΔH and ΔS suggests that **particular cons... and different interactions** occur within the binding site.

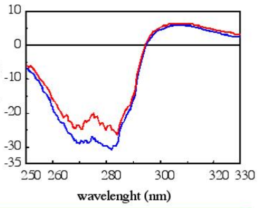
Conformational change

...UV difference spectroscopy, OBP-1F shown to undergo a ...national change upon odorant binding. The measurement ...ns were saturable and depend on ligand affinity. The ...n of the spectral properties originated from side-chain shift.



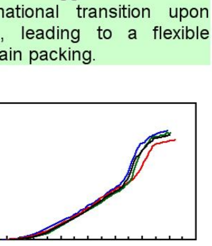
...t **perturbation difference** ...a of OBP-1F. Linalool, ...-butanone and methanol.

When added to OBP-1F, odorants induced various **spectral shifts** originated from the protein. The Scatchard plot of the spectroscopic data gave a binding stoichiometry of **1.05 ± 0,09 site per dimer**.



CD near UV spectra of OBP-1F in absence or in presence of linalool.

...**r Dichroism spectrum** ...on suggests a local ...national transition upon ..., leading to a flexible ...ain packing.

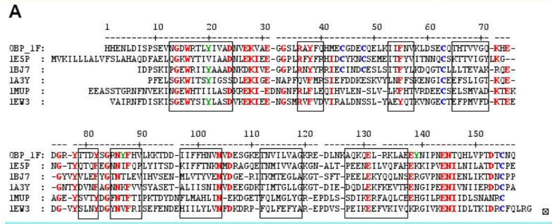


...photometric **titration** curves ...ines of OBP-1F in absence ...esence of ligands: linalool, ...-butanone and methanol.

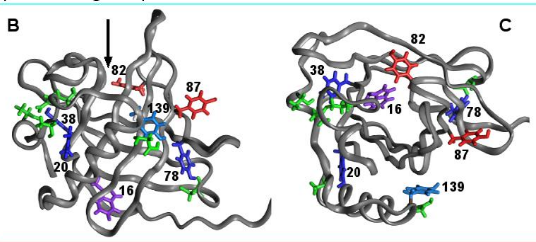
Tyrosine titration showed that two residues were affected by ligand binding and that one of the six tyrosines could never be titrated even after alkali denaturation. According to the modelled 3D structure, the residues whose pK shifted upon ligand binding could be Y20 and Y78. The untitrated residue could be Y38 because of its location in a deep crevice and the presence of two H-bonds.

Molecular modeling

A



Sequences alignment of OBP-1F with lipocalins having a known 3D structure and a sequence homology over 30% with OBP-1F. Colored residues: conserved residues, cysteines and tyrosines less or not conserved. Boxes: conserved secondary structures. PDB codes: 1E5P, hamster aphrodisin; 1BJ7, bovine lipocalin allergen Bos d 2; 1A3Y, porcine OBP-I; 1MUP, mouse major urinary protein; 1EW3, equine lipocalin allergen Equ c 1.



OBP-1F monomer 3D-structure model derived from 1EP5 coordinates. Lateral and front views (with a 90° rotation on the x-axis between each other). Arrow indicates the opening of the putative binding site and the point of view C. Numbers are relative to tyrosine and tryptophane positions in the sequence.

No tyrosyl side-chains are oriented within the binding site shaped within the ß-barrel except **Y82** present at its opening. Two tyrosines (**Y82**, **Y87**) display their hydroxyl group free to the solvent whereas the other four are buried and involved in one (**Y20**, **Y78**, **Y139**) or two (**Y38**) H-bonds with **neighbouring residues**. **Tryptophane 16** is located at the opposite side of the opening.

Conclusion

Possible properties of the tyrosines in OBP-1F accordin... modelled 3D structure. Bold numbers indicate conserved r... Dashes indicate undetermined values.

Tyrosine	location	state	H-bond	pK	pK with l...
20	strand A	buried	O-HThr146	11.2	1...
38	strand B	buried	H-NArg36 O-HThr152	-	-
78	strand E	buried	H-OCys48	11.2	1...
82	strand E	exposed	none	9.6	9...
87	strand F	exposed	none	9.6	9...
139	α helix	intermediate	O-HGlu138	9.6<pK<1...	

The **single binding site**, model supported by ITC, fluore... and difference spectroscopy assays, was postulated to be ... in **one of the two β-barrels** of the OBP-1F dimer.

The distance between the binding site and the affected r... suggests that the **side chain shifts,** shown by UV spectr... **are independent of the ligand proximity**. They are li... induced by a **conformational change**, not limited to th... environment of the reported residues which are far fro... other, but a more **wide rearrangement of the OBP-1F bac...**

The **conformational change correlated with binding** ... odorant molecule, could be involved in a mechanism ... hampers uptake of a second odorant by the other hydr... pocket for the dimeric rat OBP-1F, through a subunit cross-...

The **simulation** with molecular dynamics of **odorant dock...** the protein should allow to understand the **molecular ba... ligand binding onto OBP-1F.**

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