Characterization of protein-aroma interactions at a molecular scale

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CHARACTERIZATION OF PROTEIN-AROMA INTERACTIONS AT A MOLECULAR SCALE

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Introduction: β-lactoglobuline (β-LG) A variant

Flavour compounds of different chemical classes were tested.

The presence of 5% EtOH was proved by NMR analyses to induce no perturbation of amino acids involved in binding.

NMR spectroscopy

Bruker 500 MHz, 5 mm probe

T=37°C

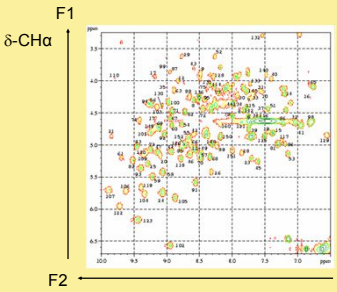
2D (^{1}H, ^{1}H) TOCSY-Watergate sequence with a mixing time of 40 ms

β-LG in 12mM NaCl buffer, pH=2.3

→ addition of an aroma compound
>50% complexed protein

β-LG → amino acids assignment [1]

NH/CHα = fingerprint region of protein



1 correlation peak
⇕
1 amino acid
δ(F2), δ(F1) in ppm

Shift variation method:
Δδ = δcomplex - δprotein

Addition of aroma compound

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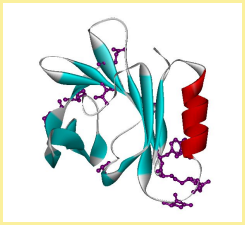
Perturbation of amino acids involved in binding

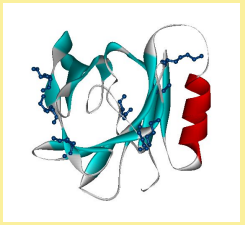
Carvone

G9, A16, I72, K75, **K101, Y102**, E108, **E127, D129, A132**



Linalool

M7, G9, D33, K70, I72, K75, K101, C121



External site | **Central cavity**

2 binding sites

FT-IR spectroscopy

FTS 6000 FT-IR spectrometer, ATR accessory

T=25°C

512 scans, resolution of 2 cm^{-1}

Analyses in 12mM NaCl buffer, 5% EtOH, pH=2

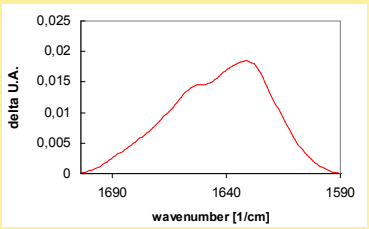
→ β-LG/aroma, >70% complexed protein

→ Aroma compound alone

Amide I band (1590-1700 cm^{-1})

→ Screening of aroma compound in function of IR profile

Amide I region of β-LG in 12mM NaCl, 5% EtOH at pH 2



Differential spectra:
Complex spectrum – aroma spectrum

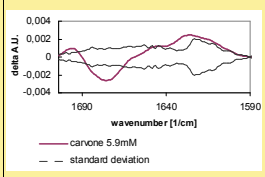
Addition of aroma compound

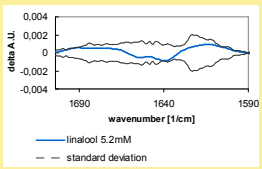
⇩

Perturbation of Amide I band

Carvone | Linalool

Differential spectrum of β-LG in presence of carvone (on the left) and linalool (on the right).
Amide I / Amide II = 1.46
Normalisation of Amide I band





Significant perturbation of Amide I band | **No significant perturbation of Amide I band**

2 binding behaviours

3D Molecular Modeling

Catalyst/Hypogen software

Point of view of the receptor

Information only from the ligand

59 aroma characterized by their affinity data K_B [2]

Atomic characteristics = Features
→ Hydrogen bond acceptor and Hydrobic

Identification of common structure of aroma having a probable common binding site

59 aroma

⇩

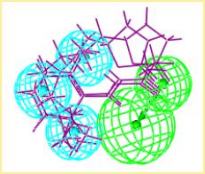
Generation of hypotheses
→ Set of features in 3D space
→ Estimation of affinity data

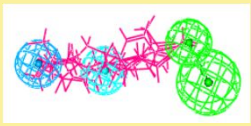
⇩

Correlation $K_{Bestimated}$ / $K_{Bexperimental}$
Alignment of aroma on the features
Analyse cost

⇩

Valid model | Division into pools





Compact alignment

Compact molecules

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External site

Extended alignment

Long acyl chain

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Central cavity

2 predictive models

Conclusion: Whatever the technique used, results confirmed the **existence of at least two different binding sites for aroma compounds on β-LG** : one binding site in the hydrophobic pocket for flavour molecules with a long acyl chain, and one external site for compact compounds.

References : [1] Uhrinova, S. *et al.*, J. Biomol. NMR, 1998, 12:89.
(2) Sostmann, K and Guichard, E., Food Chemistry, 1998, 62:509.

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