



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

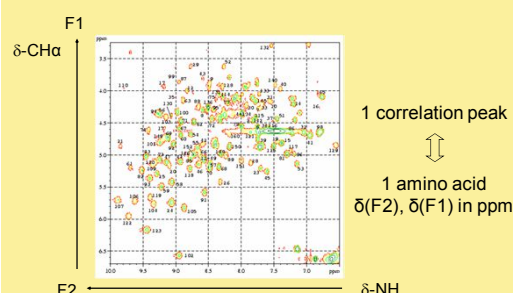
2D (^1H , ^1H) TOCSY-Watergate sequence with a mixing time of 40 ms

β -LG in 12mM NaCl buffer, pH=2.3

→ addition of an aroma compound
→ >50% complexed protein

β -LG → amino acids assignment ⁽¹⁾

NH/CH α = fingerprint region of protein



Shift variation method:

$$\Delta\delta = \delta_{\text{complex}} - \delta_{\text{protein}}$$

Addition of aroma compound

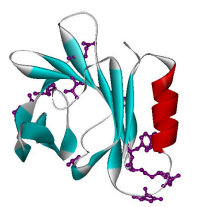
Perturbation of amino acids involved in binding

Carvone

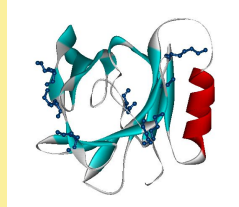
Linalool

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

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

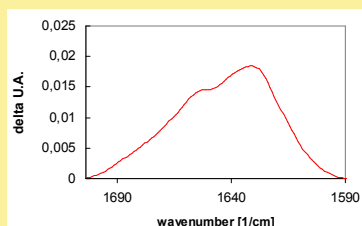
→ β -LG/aroma, >70% complexed protein

→ 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

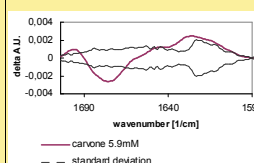
Addition of aroma compound

Perturbation of Amide I band

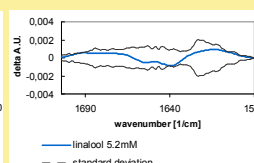
Carvone

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 Hydrophobic

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

59 aroma

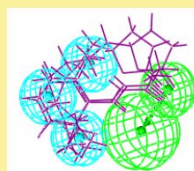
Generation of hypotheses

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

Correlation $K_{\text{Bestimated}} / K_{\text{Experimental}}$
Alignment of aroma on the features
Analyse cost

Valid model

Division into pools



Compact alignment

Compact molecules

External site



Extended alignment

Long acyl chain

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.