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Interaction of β-lactoglobulin with small hydrophobic ligands

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Introduction

Combination of **bioactive food constituents** and milk proteins with high nutritional value

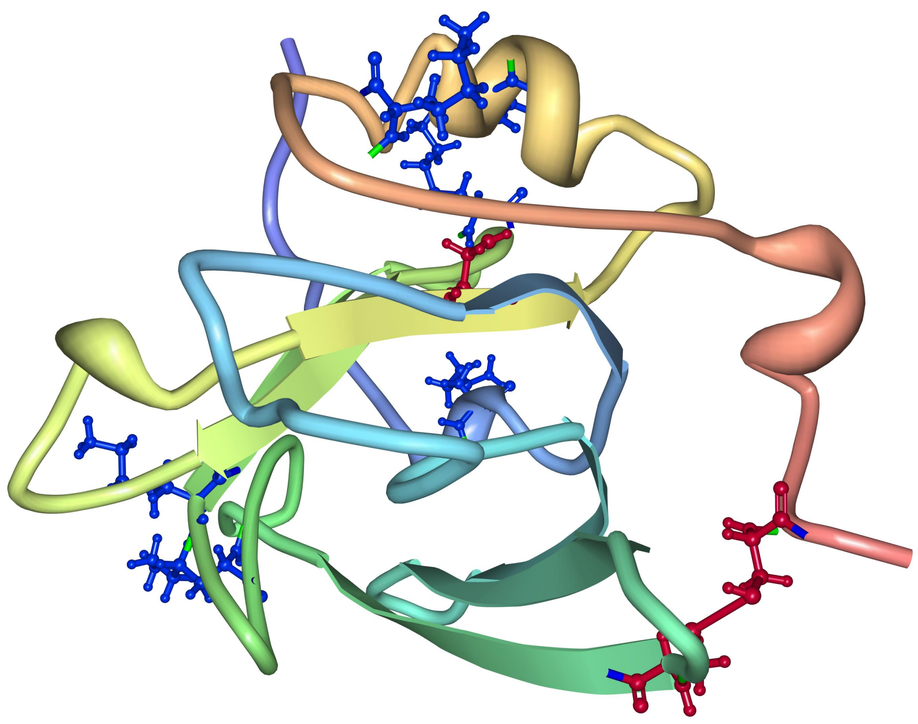
= creation of **natural and safe nanocarriers** in food.

The possibilities are explained below, using **ß-Lactoglobulin** (ß-LG) as example because ..

- pH-resistant (to pH 2).
- heat stable (to 70 °C).
- reaches the small intestine native and intact [1].

Covalent modification of ß-LG at pH 8.5

5 binding sites | 9 possible targets

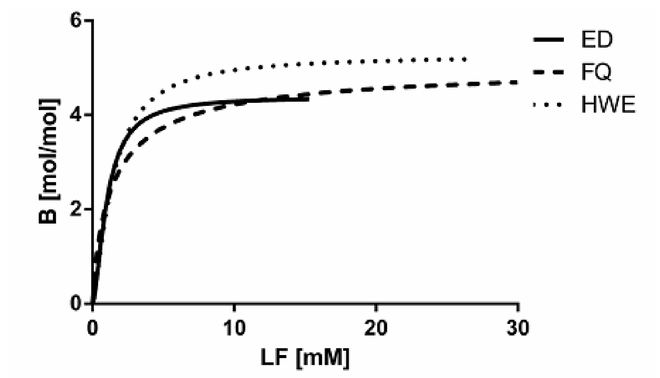


Lys14	Lys 100	Cys66
Lysi91	Lys 135	Cys121
Lys83	Lys141	Cys160

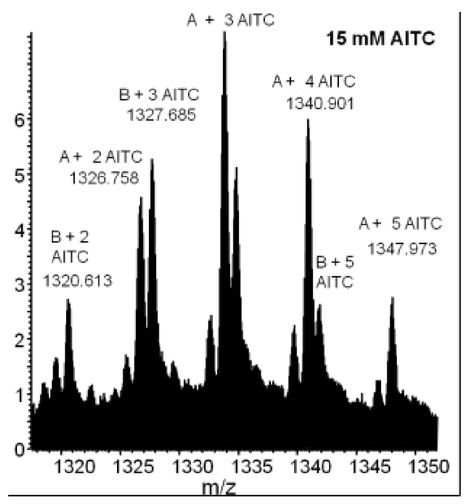
Quantitative

Comparison of standard methods with MS

Standard lab equipment methods fluorescence quenching (FQ), equilibrium dialysis (ED) and headspace-water equilibrium (HWE) all show 5 bound (B) molecules on 1 ß-LG molecule:



LC-ESI MS confirmed 5 binding sites B on ß-LG for AITC:



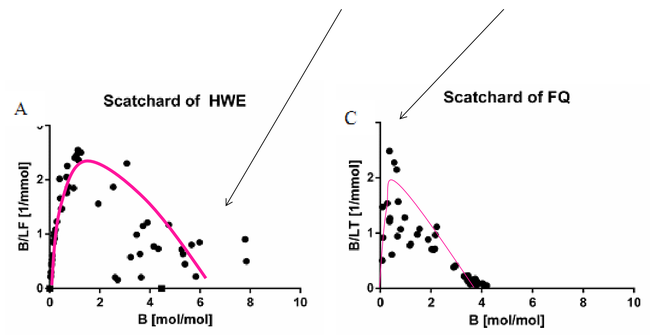
Conclusion

Covalent binding can be correctly assessed with standard lab equipment

Qualitative

Investigation of binding kinetic

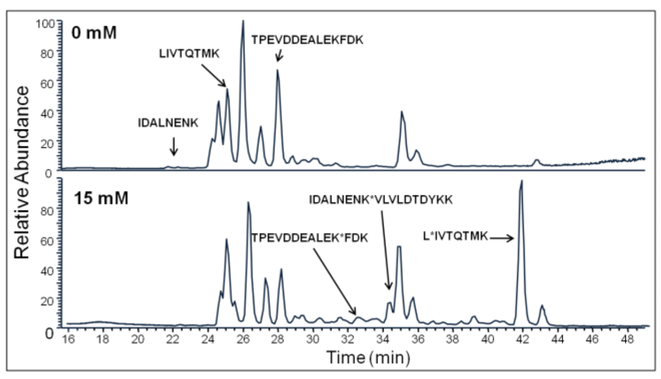
Curved Scatchard plots reveal a positive cooperative binding mode. HWE shows the greatest scatter at high B, FQ at low B.



Tryptic digestion LC-ESI MS/MS

Tryptic digestion of ß-LG and ß-LG + AITC revealed 9 possible binding sites.

Of those **2 Cys** and **3 Lys** are modified by AITC.



Conclusion

AITC binding changes proteins tertiary structure in positive cooperative manner

IMPLICATIONS

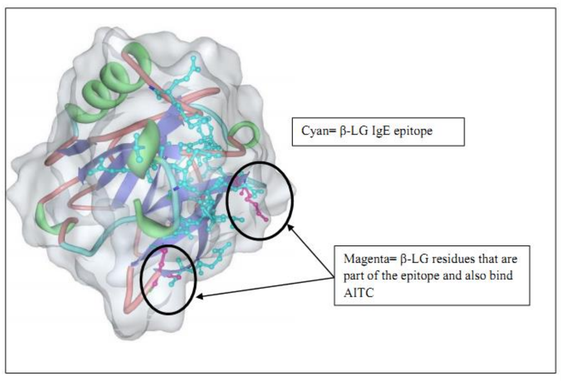
Sensory evaluation

GLDIQK production increases during tryptivc digestion of AITC modified ß-LG

Covalent modification for reducing Allergenicity?

ß-LG binds AITC at its epitope – Imunglobulin E recognition site.

This can result in a reduced allergenicity



Covalent modification for bioactive peptide generation

GLDIQK production increases during tryptivc digestion of AITC modified ß-LG

[1] Mahe, S., Messing, B., Thuillier, F., Tome, D. (1991): Digestion of bovine milk proteins in patients with a high jejunostomy. In: The American Journal of Clinical Nutrition 54 (3): 534–538.
[2] Mousavi, S., Bordbar, A., Haertle, T. (2008): Changes in Structure and in Interactions of Heat-Treated Bovine β-Lactoglobulin. In: Protein & Peptide Letters 18 (8): 818–825.
[3] Zimet, P., Livney, Y. D. (2009): Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for [omega]-3 polyunsaturated fatty acids. In: Food Hydrocolloids 23 (4): 1120–1126.
[4] Pripp, A. H., Vreeker, R., van Duynhoven, J. (2005): Binding of olive oil phenolics to food proteins. In: Journal of the Science of Food and Agriculture 85 (3): 354–362.

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