Magnetic aptamer-based oligoprecipitation as innovative sample treatment strategy for food allergen determination: egg white lysozyme as case study

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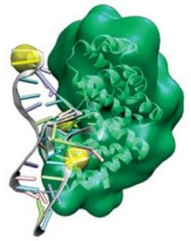
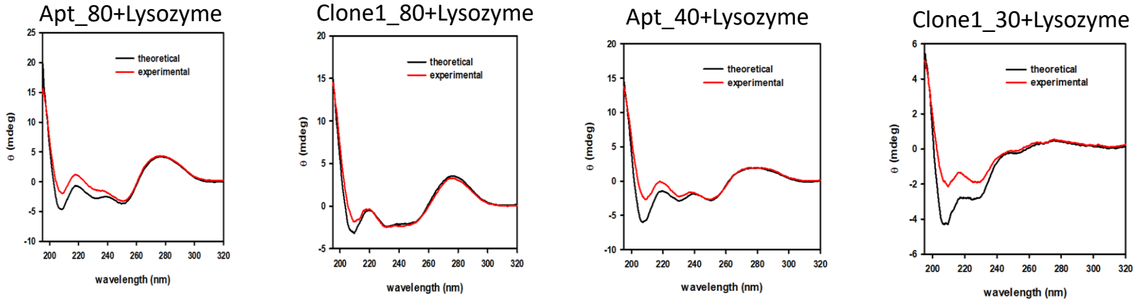
Lysozyme from chicken egg-white is known as one of the most important **allergens** to date, especially in infants and children. An innovative sample treatment strategy involving **magnetic aptamer-based oligoprecipitation** of target proteins from complex food extracts for efficient protein extraction is being developed to ensure liquid chromatography-tandem mass spectrometry-based determination of hidden lysozyme in processed foods. For this purpose, the potential of aptamer oligonucleotides as recently discovered recognition elements alternative to antibodies is being studied.

Aptamer selection

Aptamers ➡ Sequences selected from literature

Name	Sequence (5' – 3')	K_D
Apt_80 Apt_40	AGCAGCACAGAGGTCAGATGGCAGGTAAGCAGGCGGCTCA CAAAACCATTCGCATGCGGCCCTATGCGTGCTACCGTGAA	2.8 ± 0.3 nM [1]
Clone1_80 Clone1_30	GGGAATGGATCCACATCTACGAATTCATCAGGGCTAAAGAGT GCAGAGTTACTTAGTTCACTGCAGACTTGACGAAGCTT	0.46 ± 0.04 µM [2]





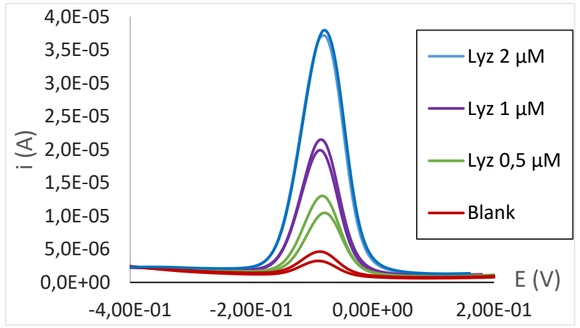
Circular dichroism (CD) spectra were acquired as preliminary assessment of lysozyme-aptamer interaction. Protein and aptamer solutions were at the same concentration (10 µM)

Magnetic beads functionalization

Electrochemical assay

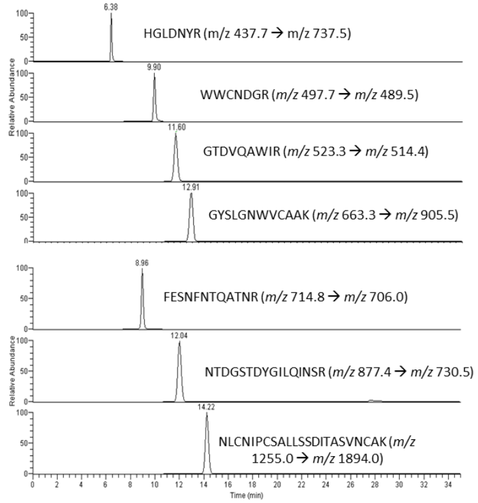
Aptamer immobilization (at 0.5, 1 and 2 µM) on the surface of carboxyl-functionalized micro magnetic beads using a (5')-amino-C6 modified and (3')-biotin-labelled sequences

Signal increase corresponding to a concentration attributable to a greater occupation of the available sites



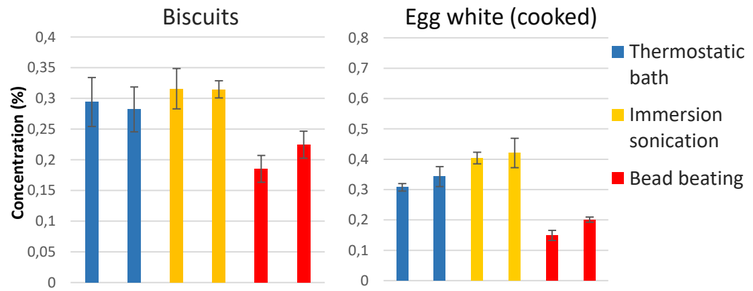
Target LC-MS/MS method for lysozyme

Unique lysozyme peptides were selected by DDA analysis and a target LC-MS/MS method was developed for lysozyme analysis



Protein extraction

Necessity to assure maintenance of lysozyme structure without compromising the interaction with the aptamer receptor, avoiding the denaturing conditions commonly used in incurred materials and processed food analysis



Sonication probe

Beat-beater

Bradford assay (280 nm)

Protein extraction carried out using a thermostatic water bath, beat beater and immersion probe sonication, testing two buffer solutions: TrisHCl 20 mM pH=8.2 and ammonium bicarbonate 50 mM pH=8

Future perspectives

Selectivity of interaction between selected aptamers and lysozyme has to be verified in presence of different interfering compounds. Non-denaturing extraction methods will be optimized using experimental design techniques. In the end, final evaluations will concern compatibility between extraction method and lysozyme isolation by the aptamer. The approach also paves the way for protein extraction involved in emerging aptasensing platforms.

[1]Han B. *et al.*, J. Chromatogr. B (2012) 903:112-117; [2]Kirby R. *et al*, Anal. Chem. (2004) 76:4066-4075