Characterization of peptides via Capillary Electrophoresis coupled with Ion Mobility Mass Spectrometry

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Introduction and context

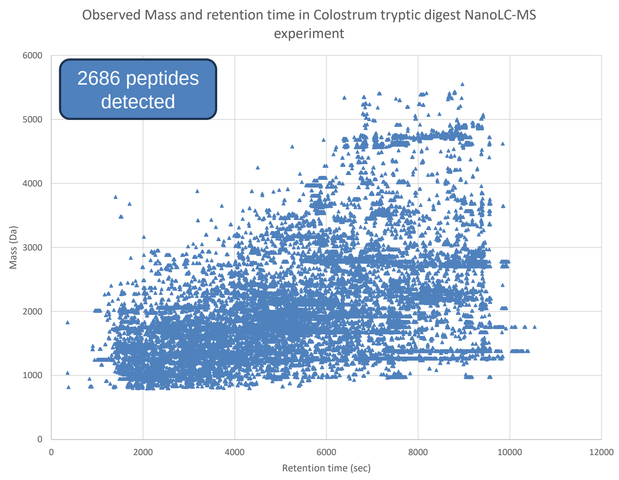
Wallonia Region's Peptiboost project focuses on the valorization of protein-rich byproducts from the agri-food industry as a source of bioactive peptides for food and feed. The characterization of the generated peptides after enzymatic digestion in native conditions without non-compliant additives in regard of food safety, or from fermentation requires novel analytical approaches. Capillary electrophoresis (CE) coupled with ion mobility spectrometry (IMS) and mass spectrometry (MS) was investigated as an alternative technique to liquid chromatography (LC)-MS for the comprehensive characterization of peptides in terms of peptides sequences and higher order structures.

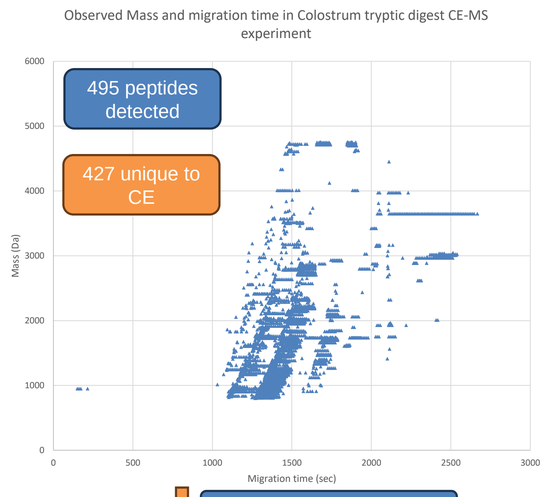
The higher order structures of peptides in solution under physiologically relevant conditions determine their biological activities. In contrast to LC-MS, CE achieves separation of ionizable compounds in denaturing or non-denaturing and physiologically relevant conditions based on their average charge in solution (depending on the pH of the background electrolytes) and their hydrodynamic radius (that is shape dependent). The separation of highly hydrophobic and hydrophilic compounds is achievable by CE while providing insights into the structural characteristics of analytes in solution.

Ion mobility coupled to mass spectrometry (IM-MS) detection operates in the gas phase, providing additional dimensions of separation to the upstream separation methods, including LC or CE. Ion mobility provide structural parameters in the form of the Collision Cross Section (CCS) and mass spectrometry (MS and MSMS) provides m/z and stoichiometry, sequence of peptides, resilience to the fragmentation (breakdown curves) and the resilience of the structure in the gas phase under soft collision activation (collision induced unfolding).

CE was hyphenated in-line with ion mobility mass spectrometer using sheath liquid or sheathless interfaces.

Bovine Colostrum proteomics NanoLC vs CE





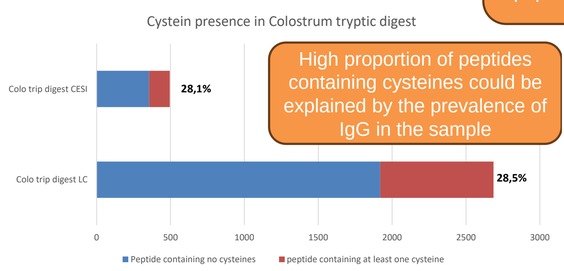
Injection : ~0,5µg of peptide

Injection : ~5ng of peptides

Peptide detection was performed using "fragpipe" software [1]

5612 ions selected for MS² not identified by the detection software
➔ Potentially much more peptides not recognized by the algorithm

NanoLC-MS experiments were performed using a reversed phase C-18 column coupled with a Thermo Q-Exactive operating in ion positive mode from m/z 300 to 1750.
CE-MS Experiments performed on a SCIEX Beckman CESI8000 equipped with an OptiMS bare fused silica capillary cartridge operating in normal polarity (+30kV) in 10% acetic acid as BGE, coupled using a modified SCIEX sheathless interface to a Thermo Q-Exactive operating in ion positive mode from m/z 300 to 1750. In both cases, MS/MS were performed on top 12 most intense peaks at normalized collision energy (NCE) set to 35



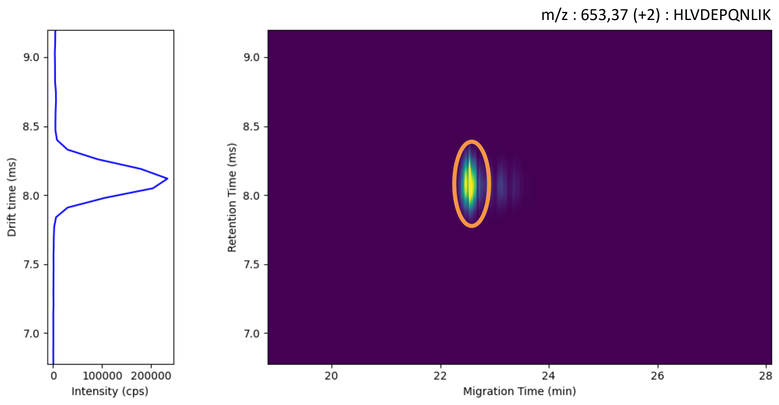
High proportion of peptides containing cysteines could be explained by the prevalence of IgG in the sample

BSA Typtic Peptides Conformation in the liquid (CE) and gas phase (IMS)

Case 1 :
- **1 peak in CE**
- **1 peak in IMS**

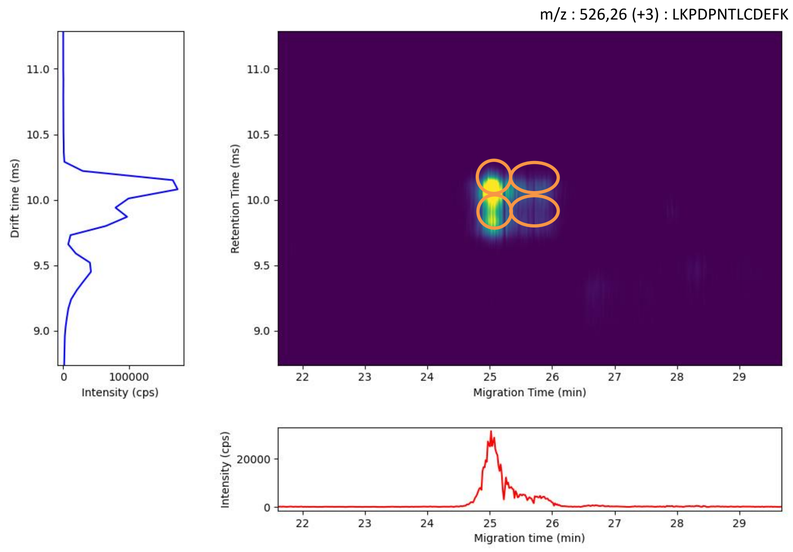
➔ Expected

Observed for most peptides



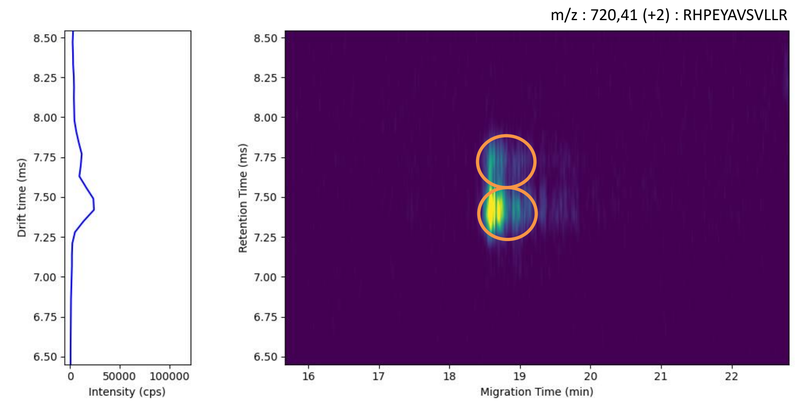
Case 2 :
- **2 peak in CE**
- **2 peak in IMS**

1 peak in CE ≠ 1 peak in IMS



Case 3 :
- **1 peak in CE**
- **2 peak in IMS**

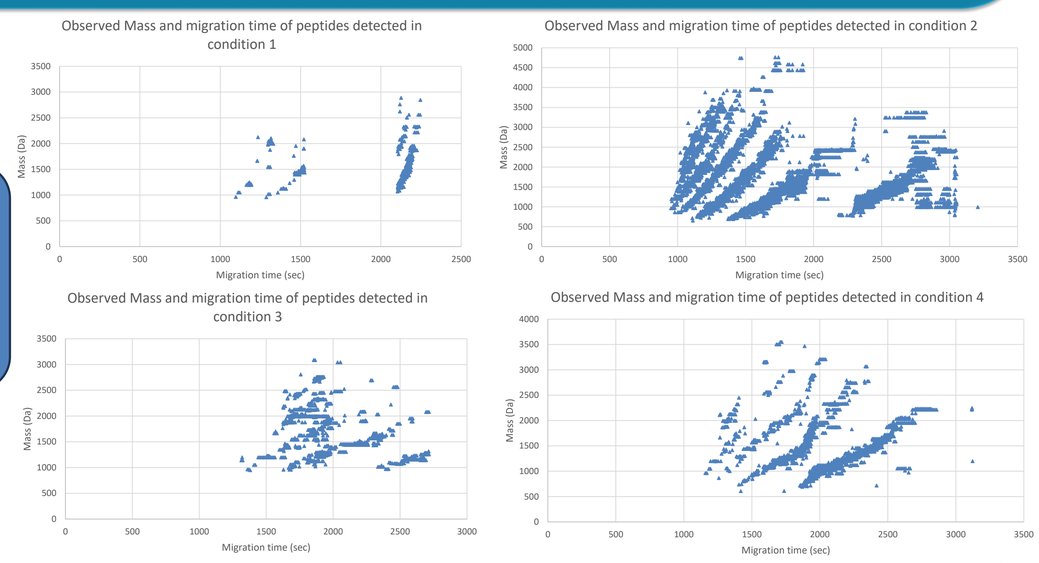
Fast equilibrium in solution or conformationnal change during ionization process ?



Experiments performed on a Beckman-Coulter P/ACE MDQ Capillary electrophoresis system operating in normal polarity (+30kV) using bare fused silica capillary in 1% formic acid as BGE, coupled using an inhouse sheath-liquid interface to a waters Synapt G2 HDMS operating in positive ion mode from m/z 150 to 2000.

For small peptides (<20 AA), the conformation observed in the gas phase may no be directly correlated with the conformation observed in solution. Thus, precaution must be taken when drawing conclusion about a peptide conformation based solely on gas phase data.

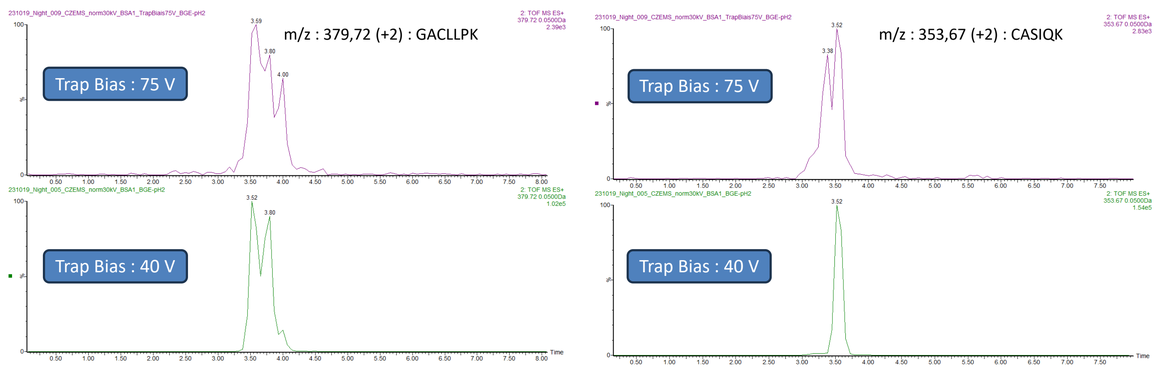
Distribution of mass and migration time of peptides detected in reduced and alkylated Colostrum peptide hydrolysates under different conditions



Parameters tested for hydrolysis include :
- Enzyme/subtract ratio
- Preheating of the sample before digestion
- Digestion time
- Enzyme deactivation method (pH or temperature)

As expected, slight differences in hydrolysis condition leads to differences in peptides length and variety that can be easily screened using CE-MS experiment.

CIU of BSA tryptic peptides



The conformation of peptides in the gas phase could be affected by the uptake of internal energy due to collision with neutral gas in the ion optics.
Here we intentionally softly increase the collision energy before the separation of ionized peptides by the ion mobility cell.
Additional arrival time distribution (ATD) could be induced (see examples here) while several ATD can merge due to CIU (data not shown).
Correlation between the mobility peaks in solution (CE) and in the gas phase (IMS) have to be wisely accessed.
CIU might be used as a supplementary fingerprinting tool for peptide identification.

Acknowledgment
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References
[1] https://fragpipe.nesvilab.org