Structural characterization of proteins by using enzymatic reactor

E.Grifnée[1], G.Mazzucchelli[1], N.Smargiasso[1], E. Hanozin[1], F. Baumans[1], C. Hage[2], A.Sinz[2], L.Quinton[1] and E.Depauw[1]

[1]Mass spectrometry laboratory, GIGA-R University of Liège [2]Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin-Luther University of Halle-Wittenberg, Germany

Contact : elodie.grifnee@ulg.ac.be

Overview

Purpose : Structural characterization of proteins by using enzymatic reactor coupled to mass spectrometry.

Tools : Enzymatic reactor coupled to mass spectrometry, traveling-wave ion mobility spectrometry (TWIMS), chemical cross linking.

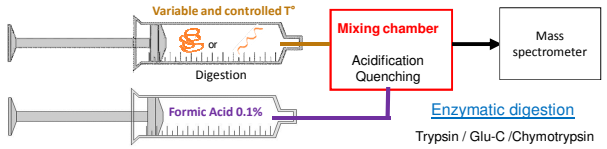
Main results : - Information on the accessibility of peptides to digestion in native conditions
- Presence of both structured and deployed protein domains during digestion.

Introduction

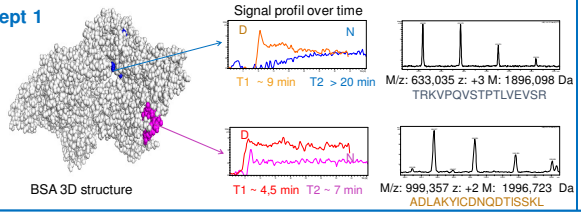
The tridimensional structure of proteins and the mapping of protein-protein interactions are precious sources of information for the understanding of their function. The goal of this study is to obtain structural information concerning native, partially or completely denatured proteins or interacting with a ligand using digestion kinetic data. Our work hypothesis is based on the observation of digestion kinetic variation (peptide nature and digestion rate) resulting from protection effects by comparing digestion in native or denatured conditions. Protection of cleavage zones can be induced by intermolecular interactions (complexes formation) or by intramolecular interactions (protein folding). Special attention regarding residual structure conservation for protection effect must be considered when using peptides apparition times.

In parallel, chemical cross-linking mass spectrometry (CX-MS) methods are currently used to probe the protein-protein interaction area. Here we first investigate the 3D structure of the well-known Cab-HuL6 Lysozyme complex based on CX-MS.

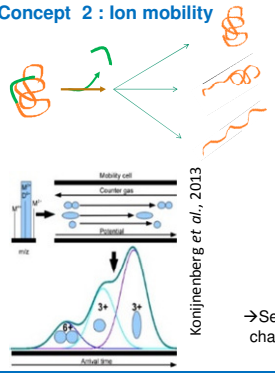
Enzymatic reactor

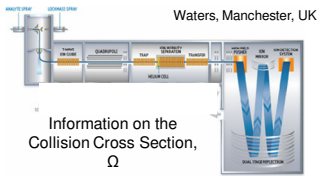


Concept 1



Concept 2 : Ion mobility



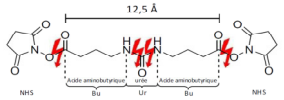


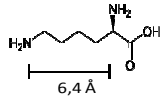
Mobility law: $v = KE$

Mason-Shamp law: $K = \frac{3}{16}\left[\frac{2\pi(m+M)}{mMkT}\right]^{1/2}\frac{ze}{N\Omega}$

→Separation of ions based on their size, shape and charge under the influence of an electric field and a buffer gas.

Cross linker





Target residues :

K and N-terminus (also S,T and Y)

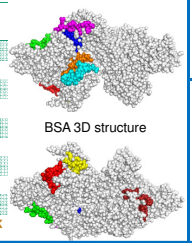
= CID cleavable bonds

Lysines K have a dynamic lateral side chain length of 6,4 Å

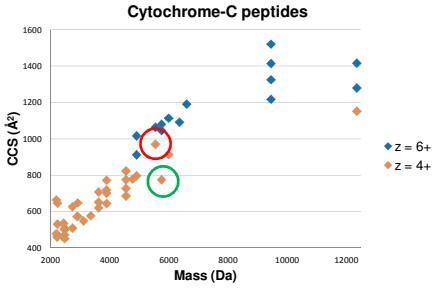
Maximum distance (α) between two lysines K : Cα = 25,3 Å

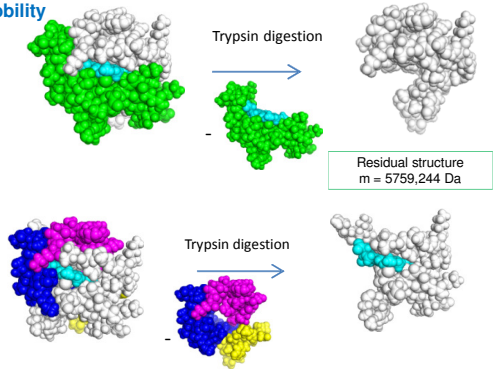
Results part 1 : Structural characterization thanks to online digestion

m/z	Native Time	Denatured Time	3D Structure	Sequence
633,04	20	9,2	Buried	TRKVPQVSTPTLVEVSR
756,284	7,3	5,3	Surface	TPDETYVPKAFDE
831,473	23,7	7,1	Surface	DKLKHLVDEPQNLI
837,312	6,9	4,7	Surface	TKCCTESL-RPCFSAL
839,79	7,5	5,7	Surface	CVLHE-VTKCCTESLV
918,34	6,1	4,9	Surface	CVLHEK-KVTKCCTESL
982,052	25,5	11,8	Buried	CKSHGDLLECADDRADLAK
999,369	6,7	4,5	Surface	ADLAKYICDNQDTISSKL
1080,4	6,9	4,7	Surface	RETYGDMADCCEKQEPER
1242,459	6,9	4,9	Surface	QEPERNECFLSHKDDSPDLPK



Results part 2 : Residual structure analyses by ion mobility

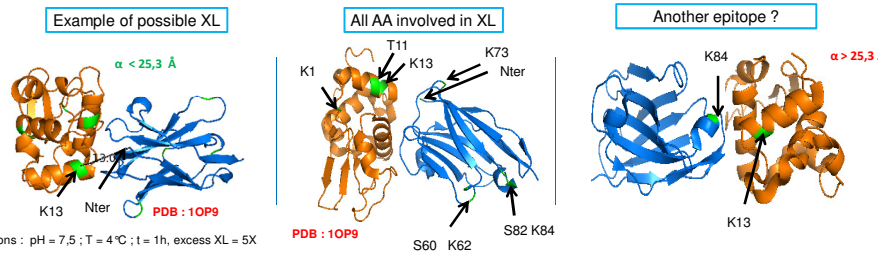




Loss of structuring domains or total destructuration ?

Results part 3 : Study of Cab-HuL6 Lysozyme complex[1]

[1] M. Dumoulin *et al.*, Nature, 2003.



Conditions : pH = 7,5 ; T = 4 °C ; t = 1h, excess XL = 5X

- Non-specific interactions between **cAb-HuL6** and **lysozyme** ?
- Most CX residues are located on loops and flexible elements. The flexible character of these parts may allow the access to CX positions theoretically forbidden based on distance restrains.

Conclusions

1.Thanks to online enzymatic digestion on native and denatured protein coupled to mass spectrometry, it is possible to obtain information on the accessibility of peptides to digestion in native conditions. From those data it is possible to locate peptides in the protein 3D structure.

2.Ion mobility (IMS) analyses of residual structures of protein allowed to highlight the presence of both structured and deployed protein domains. However, IMS only gives insights into ion density but not the actual degree of protection. Therefore, an independant characterization technique is required to further confirm these preliminary results.