Capillary Electrophoresis and Ion Mobility coupled to Mass Spectrometry as complementary tools for cysteine connectivity identification in peptides

Cédric Delvaux[1] and Philippe Massonnet[1], Christopher Kune[1], Gregory Upert[2], Gilles Mourier[2], Jean R.N. Haler[1], Nicolas Gilles[2], Loic Quinton[1], Johann Far[1], Edwin De Pauw[1]

[1]Laboratory of Mass Spectrometry, University of Liege, Allée du six aout, 11 - B6c, Quartier Agora, 4000 Liège, B-4000 Liege, Belgium [2]Commissariat à l'Energie Atomique, DSV/iBiTec – S/SIMOPRO, F91191 Gif-sur-Yvette, France

Contact : c.delvaux@ulg.ac.be

Overview

1) **Purpose:** Combination of ion mobility and capillary electrophoresis techniques to perform the separation and characterization of peptides bearing 2 intramolecular disulfide bonds in the gas phase and in solution
2) **Methods:** Traveling-wave ion mobility spectrometry (TWIMS) and capillary zone electrophoresis coupled with mass spectrometry (CZE-MS) for the experimental approach. Combination of molecular mechanics and protonation prediction for the theoretical approach.
3) **Results:**
   - Partial but sufficient separation was obtained by TWIMS depending on the studied charge state
   - Baseline separations were obtained using CZE-MS on all studied peptides, sometimes after a background electrolyte pH optimization
   - Theoretical predictions were used to calculate the precise in solution net charge, allowing the prediction of the migration behaviors of the different disulfide isomers in solution and in the gas phase

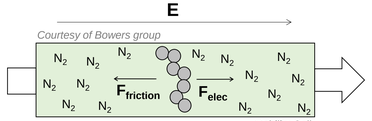
Introduction

Oxidation of cysteines leading to disulfide bond formation is an important biological process implied in peptide/protein folding. Disulfide bonds are post-translational modifications (PTMs) involved in specific folding formation by providing the biologically active conformation of numerous peptides and proteins. Associated with other structuring phenomena, disulfide bridges allow the folded species to efficiently act on their biological targets(1). The characterization of the disulfide bond connectivity is still an analytical challenge for such structured peptides/proteins, especially when the relative amount of sample is limited. The purpose of this study is to compare and develop new and fast in solution (Capillary Electrophoresis) and gas phase (Ion Mobility Spectrometry) strategies coupled to Mass Spectrometry to characterize disulfide bond connectivities. Finally, theoretical calculations were undertaken to predict the migration behaviors in both the gas phase and in solution.

(1) Gongora-Benitez , Tulla-Puche J., and Albericio F. (2014) Multifaceted Roles of Disulfide Bonds. Peptides as Therapeutics. Chemical Reviews 114, 901−926

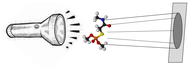
Materials and Methods

Separation in the **gas phase** according to both charge (**q**) and collision cross section (**Ω**)



$$K = \frac{3q}{16N}\cdot\left(\frac{2\pi}{kT}\right)^{\frac{1}{2}}\cdot\left(\frac{m+M}{m.M}\right)^{\frac{1}{2}}\cdot\frac{1}{\Omega}$$

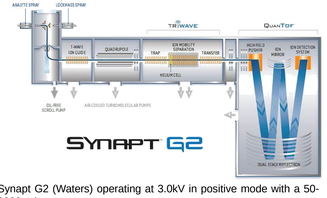
Collision Cross Section (**Ω**) :



System CGS

K: mobility in gas phase (cm²/(V.s))
q: charge of the ion (esu)
k: Boltzmann's constant (1.381 erg.K⁻¹)
m: mass of buffer gas (g)
N: density number of buffer gas
T: temperature (K)
M: mass of ion (Da)

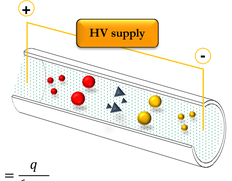
Ω: Collision Cross Section (cm², Å²) is accessible through a calibration



Synapt G2 (Waters) operating at 3.0kV in positive mode with a 50-2000 m/z mass range

TWIG: Transfer : 0,5 mbar - IMS cell : 1,68 mbar - Trap : 0,5 mbar. The waves parameters were separately optimized for each peptide group

Separation in **solution** according to both charge (**q**) and hydrodynamic radius (**r**)



$$\mu_e = \frac{q}{6\pi\eta r}$$

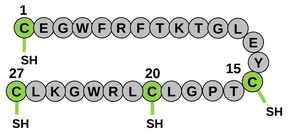
μ_e: Electrophoretic mobility (m²/(V.s))
q: charge of the ion (C)
η: viscosity (kg/m.s)

r: hydrodynamic radius (m)

CE: CESI 8000 (Sciex Separation) operating at +30kV on a bare fused silica 90cm x 150µm (OD) x 30µm (ID) in formic acid or ammonium acetate as background electrolytes. The CESI8000 was coupled to MS though a sheath liquid interface (Analis SL CE-MS Sprayer, ref. PN 10-301347) supplied with appropriate sheath liquid delivered at 1µL.min⁻¹
MS : LTQ-FT Thermo (IT-FT ICR 50k resolution) operating at 2.0kV in positive ion mode with a 150-2000 m/z mass range

Results : experimental section

Model synthetic peptide (3161.49 Da):

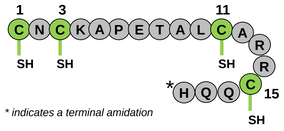


1) ModBea (synthetic) : $Cys_1 – Cys_{15}$ / $Cys_{20} – Cys_{27}$

2) ModGlo (synthetic) : $Cys_1 – Cys_{20}$ / $Cys_{15} – Cys_{27}$

3) ModRib (synthetic) : $Cys_1 – Cys_{27}$ / $Cys_{15} – Cys_{20}$

Apamin (2025.88 Da) :



** indicates a terminal amidation*

1) ApaBea (synthetic) : $Cys_1 – Cys_3$ / $Cys_{11} – Cys_{15}$

2) Apamin (natural) : $Cys_1 – Cys_{11}$ / $Cys_3 – Cys_{15}$

3) ApaRib (synthetic) : $Cys_1 – Cys_{15}$ / $Cys_3 – Cys_{11}$

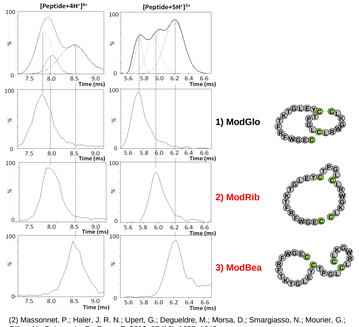
Conotoxin (1301.46 Da) :

1) Conotoxin α (synthetic) : $Cys_1 – Cys_7$ / $Cys_2 – Cys_{11}$

2) Conotoxin χ (natural) : $Cys_1 – Cys_{11}$ / $Cys_2 – Cys_7$

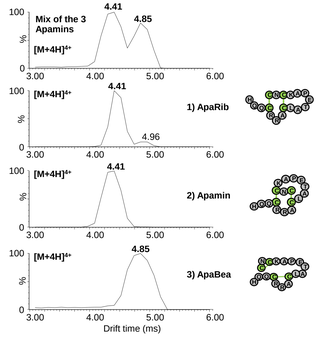
NB: The last possible disulfide connectivity was not synthesized due to the vicinal disulfide bond between Cys_1 and Cys_2

IM-MS(2)

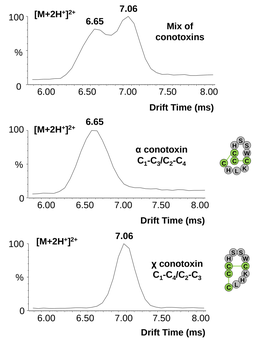


(2) Massonnet, P.; Haler, J. R. N.; Upert, G.; Degueldre, M.; Morsa, D.; Smargiasso, N.; Mourier, G.; Gilles, N.; Quinton, L.; De Pauw, E. **2016**, *27* (10), 1637–1646.

IM-MS



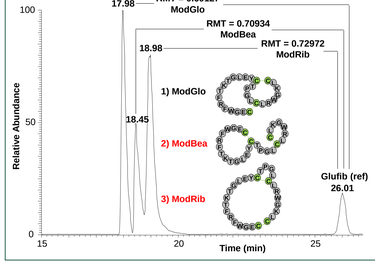
IM-MS



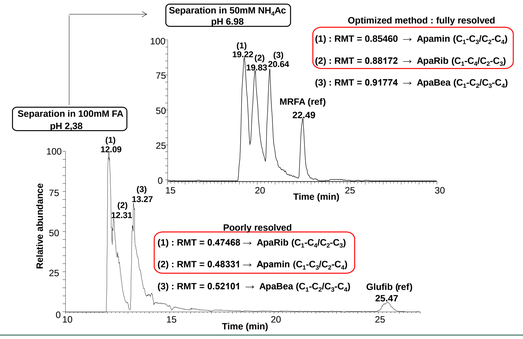
CZE-MS

Separation in 10% Acetic Acid + 20% IsoPropanol

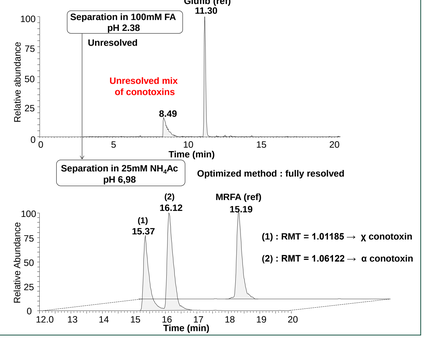
Step 1) Relative migration times (RMT) were determined on each disulfide isomer separately to a reference (Glufib) : **RMT = MT peptide / MT GluFib** (n=6)
Step 2) Migration of a mixture of the 3 disulfide isomers and Glufib (reference) :



CZE-MS



CZE-MS



Results : theoretical section

IMS: CCS estimation from structure optimization

Non optimized folding for each disulfide connectivity

Molecular Mechanics
AMBER in vacuo
Structure optimization for the different charge states

Optimized geometry for each disulfide connectivity → IMOS N_2 PA/TM/EHSS → **CCS calculation**

CE: μ_e estimation from structure and charge state optimization

Charge state distribution evaluation from "linear structure"

Non optimized folding for each disulfide connectivity

Molecular Mechanics
AMBER CPCM water
Structure optimization for all possible charge states

Optimized geometry for each disulfide connectivity

Molecular Mechanics
AMBER CPCM water
structure reoptimization for the most probable charged residue

H++ computation (3)
Protonation state energies calculation

Computed pKa shift induced by the folding

NO

Stable pKa shift ?

YES

Set of optimized geometries → Gyration radii determination → Final gyration radius ponderation by in-solution charge states

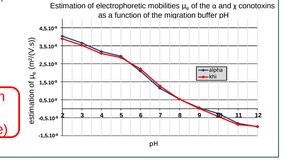
Final gyration radius ponderation by in-solution charge states → Form factor evaluation (Compactness surface/volume ratio, sphericity (Ψ)) → Rg/Rh factor conversion

μ_{EOF}

μ_e estimation

μ_e estimation for each pH value (MS buffer compatible)

(3) http://biophysics.cs.vt.edu/



Conclusions and Acknowledgments

- CZE-MS and IM-MS are complementary techniques to assign the disulfide bridges connectivity in peptides
- CZE-MS yields structural information about conformations in solution while IM-MS yields structural information in the gas phase
- Theoretical calculations support the experimental observations

- Analis (Suarlée, BE) and its R&D department are acknowledged for hardware and technical support
- This project is also funded by a the F.P. 7 VENOMICS European project