Evaluation of dansyl peptides MS and MS/MS features to enhance the LC-MALDI-MS/MS analysis

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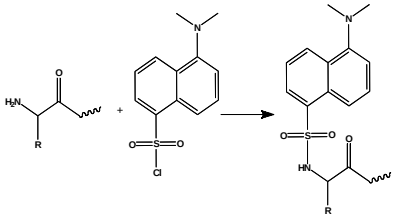
Chiappetta Giovanni[1,2], *Ndiaye Sega*[1], *Demey Emmanuelle*[1], *Haddad Iman*[1], *Marino Gennaro*[2], *Amoresano Angela*[2], *Vinh Joëlle*[1]

1: Ecole Supérieure de Physique et de Chimie Industrielles de Paris, 2: Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II

INTRODUCTION

MALDI-MS development is one of the events playing a key role in the implementation and the appraisal of the proteomics approach.
Despite the fact that MALDI ionization mechanisms are not yet completely understood, it is well accepted that ionization efficiency is highly dependent on peptide structure and amino acid composition (like presence of basic sites and hydrophobic groups (1)). Hence, in order to increase MALDI-MS interest in proteomics, many studies based on peptides chemical modification with "MALDI-active" groups were realized (2-3).

Dansyl chloride (DNS-Cl) is a fluorescent reagent, largely used in biochemistry to modify the primary amine group of proteins and peptides for N-terminal sequencing.
MALDI features of DNS-Cl peptide tagging were first evaluated by Park *et al.* (4).



Besides improvement of signal to noise ratios (S/N), they found that the typical protein MALDI-MS fingerprint changed because usually un-detected peptides were revealed with high signal intensities. For this reason improved PMF identification results from combining peak lists of tagged and native samples, separately analyzed.
In the present work, effects of DNS-Cl labelling on MALDI-MS peptide analysis are explored and its advantages for LC-MALDI-MS/MS are also evaluated.

METHODS

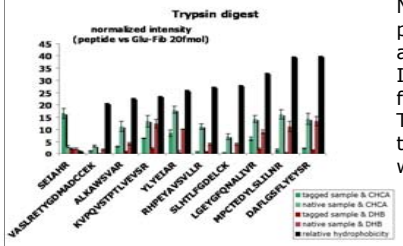
•Trypsin, chymotrypsin and Glu-C digestions were carried out using an enzyme/substrate ratio of 1:50 (w/w) by microwave heating in a water-bath for 1 minute in a domestic microwave oven at 700W.

• 50µL of DNS-Cl (18.5nmol/µL in ACN) were added to 50 µL of protein digest (2pmol/µL). Reaction was carried out warming tubes in a water-bath for 5 min in a domestic microwave oven at 700 W.

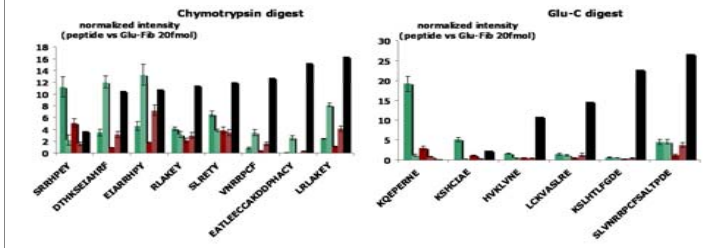
•NanoLC-MALDI-MS/MS experiments were performed on a 4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems) coupled to an Ultimate 3000 system (Dionex). Peptide mixtures were loaded onto an Dionex reversed-phase cartridge (C18 PepMap 100, 5 × 0.3 mm, 3 µm) at 20 µL/min of solvent A (98/2/0.1, H_2O, ACN, FA) for 5 min. Peptides were then separated on a Dionex reversed-phase column (C18 PepMap 100, 150 mm × 75 µm, 3 µm), at a flow rate of 220nL/min. Linear gradient from 0 to 50% solvent B (10/90/0.1, H_2O, ACN, FA) in 35min was used. Eluent was continuously mixed with CHCA (5mg/mL, flow rate: 436nL/min) via a T-junction. 180 spots were collected on a 4800ABI plate (one spot every 10 sec) using a Probot™ microfraction collector. The resulting plate was analyzed in MS and in MS/MS mode by the 4800 MALDI-TOF/TOF mass spectrometer. The MS spectra were recorded using a fixed laser intensity of 2100, for 1200 shots. The 7 more intense peaks were selected as precursor for the successive MSMS analysis. The fragmentation spectra were acquired using a laser at 3500, for 2400 shots with an acceleration voltage of 2kV, CID OFF and the metastable suppressor ON.

RESULTS

MS features on a BSA digest: link with hydrophobicity

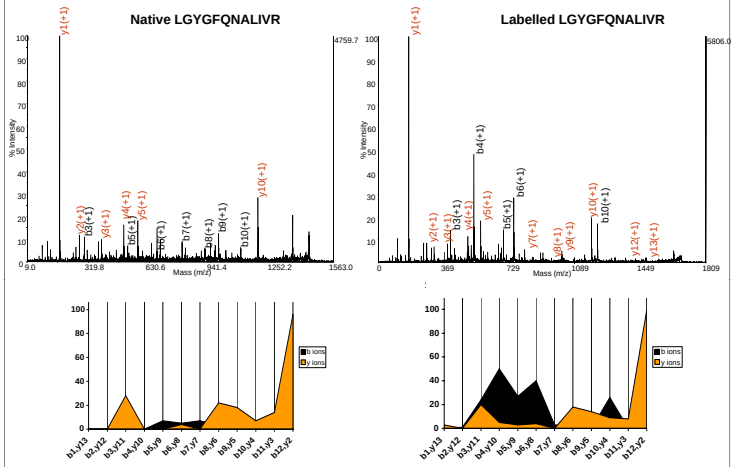


Normalized peak intensities of peptides observed both in tagged and native forms are compared. Increased signal intensities are found for hydrophilic peptides. This improvement is lower when the more hydrophilic DHB matrix was used.



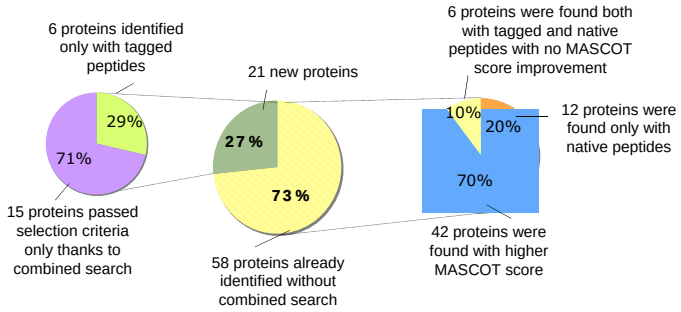
Average intensities on 10 spots

MS/MS features on a BSA digest: fragmentation efficiency



Comparing spectra from native and tagged peptides, presence of the aromatic dansyl moiety in the N-terminal position increases the generation of b-ions in this region. The complementary y-ions generation is not affected by the same effect, meaning that fragmentation efficiency may also be enhanced. The behavior showed here is independent from the precursor ion intensity.

LC-MALDI-MS/MS features on *E. coli* tryptic digest



DNS-Cl tagged and native peptide mixtures were analyzed by LC-MALDI-MS/MS in separate runs. MS/MS data of the two separate runs were combined and used for the database search by MASCOT software. Results were processed with the software MyProms (5) allowing to calculate protein score considering only the higher ion score of peptides observed both in native and tagged forms.
Combined search allows to improve LC-MALDI-MS/MS results in terms of identified proteins, MASCOT score and sequence coverage.

CONCLUSION

•Peptide amines sulfonation by DNS-Cl may have two opposite effects on MALDI ionization: increasing the peptide hydrophobicity the co-crystallization of the most hydrophilic peptides with the matrix is improved. This effect is less important for the most hydrophobic peptides that may show only the negative effect resulting by the decreased basicity after the amine derivatization.

•The behavior discussed above leads to obtain complementary data from the analysis of tagged and native peptide mixtures. Thus improved identification of the LC-MALDI-MS/MS analysis occurs if the native and tagged peptide mixtures are separately run and if the peak lists are then combined for database search.

•MS/MS spectra quality may also be improved by the increased generation of *b-ions*. This effect may be attributed to the presence of the UV absorbing dansyl moiety (λabs=334 nm) that may increase the energy transferred from laser to the analyte. This effect may induce fragmentation of the peptide bonds in the N-terminal region.

•Finally, the method developed consists of a full microwave based protocol that turns out to be very rapid and efficient.

REFERENCES

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