ENRICHMENT BY FRACTION COLLECTION DEVELOPED FOR CE/MALDI-MS TO ANALYZE PROTEINS BY TOP-DOWN STRATEGY

Michael Biacchi[1], Ricky Bhajun[1], Yannis-Nicolas François[1], Emmanuelle Leize-Wagner[1]

1 - Laboratoire de Dynamique et Structure Moléculaire par Spectrométrie de Masse (LDSM2), CNRS – UMR7177, University of Strasbourg, Strasbourg

Protein identification and characterization seem to be one of the most important challenges posed to analytical sciences these last years. Capillary electrophoresis (CE) coupled with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) is a technique highly suitable for the separation and detection of intact proteins. Since 1995, several approaches for coupling CE to MALDI-MS have been described[1,2]. However, due to its miniaturized format, CE suffers from a lower loading capacity which is a major drawback for trace analysis of proteins.

In this study, new instrumental developments allowed to improve CE-MALDI/MS coupling for the analysis of intact proteins. CE fractions were collected directly on a MALDI target, using a sheath-flow interface. A homemade delivery matrix system was developed in the laboratory to allow dry droplet deposit with matrix addition just after sample deposition. Moreover, a modification of the CE system allows obtaining a CE-UV-MALDI/MS coupling.

In a first step, a reproducible separation of protein mixture has been realized in CE/UV. Different background electrolytes and capillary coatings have been tested to allow an optimized separation compatible with CE/MALDI-MS. 1% Formic acid aqueous solution was selected and protein adsorption during CE separations was prevented by coating the capillaries with Polybrene. In a second step, we have evaluated the robustness of the homemade CE-MALDI interface. For this reason, all proteins were analyzed by MALDI-TOF-MS directly after fraction collection. In a third step, fraction enrichment has been realized by the repetition on the same MALDI target of several CE separations performed on the same proteins mixture.

The ultimate aim of this project is to obtain a suitable CE/MALDI-MS methodology for the analysis of complex proteins mixture by a Top-Down approach.

[1] *Foret F., Müller O., Thorne J., Götzinger W., Karger B.L., Journal of Chromatography A 1995, 716, 157-166.*
[2] *Walker K.L., Chiu R.W., Monnig C.A., Wilkins C.L., Analytical Chemistry 1995, 67: 4197-4202.*