Qualitative and Quantitative Analysis of Post-translational Modifications Using CESI-MS

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Introduction

Traditionally, LC-ESI-MS and/or MALDI-MS are the analytical techniques of choice for the analysis and quantification of post-translationally modified proteins and peptides. However, it is well documented that analysis of especially phospho-peptides using LC-ESI-MS is not without challenges. Poor ionization efficiency, low separation efficiency and neutral losses etc. often impedes detection and characterization. Thus, we evaluated the capability of CESI-MS for the separation and analysis of various common protein modifications.

Histones

These small highly basic proteins are subjected to extensive post-translational modifications, e.g. acetylation, phosphorylation, methylation and deamidation. Both the complexity and unique physical and chemical property of this protein family is a challenging task requiring the most advanced separation techniques and sophisticated instrumentation available. Although CESI-MS detection of histone modifications is limited by the mass loading capacity of the system, it has the advantage of providing a rapid overview of the modification status of intact proteins, allowing fast and reproducible comparisons between treated and untreated cells or different cell phases [2,3].

Methods

For the analysis of protein modifications various samples were investigated: Core histones which are known to be extensively modified, as well as phosphoproteins and phosphopeptides present in a highly complex sample, the proteome of yeast. The different samples were analysed by CESI-MS using a prototype Beckman Coulter CESI 8000 capillary electrophoresis system sold through SCIEX Separations, a part of AB SCIEX, coupled via a sheathless high sensitive porous sprayer interface to an LTQ Orbitrap XL ETD (ThermoScientific) mass spectrometer [1].

References

[1] Moini, M. Anal. Chem. 2007, 79: 4241-4246

[2] Faserl, K. et al., Anal. Chem. 2011, 83: 7297-305

[3] Sarg, B. et al., MCP. 2013, 12: 2640-2656

Acknowledgment

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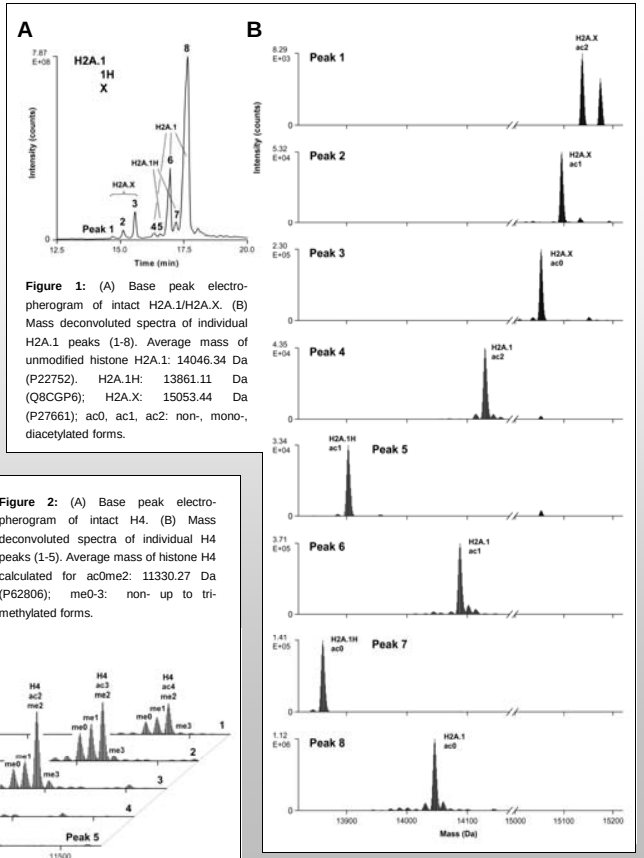

Figure 1: (A) Base peak electro-pherogram of intact H2A.1/H2A.X. (B) Mass deconvoluted spectra of individual H2A.1 peaks (1-8). Average mass of unmodified histone H2A.1: 14046.34 Da (P22752). H2A.1H: 13861.11 Da (Q8CGP6); H2A.X: 15053.44 Da (P27661); ac0, ac1, ac2: non-, mono-, diacetylated forms.

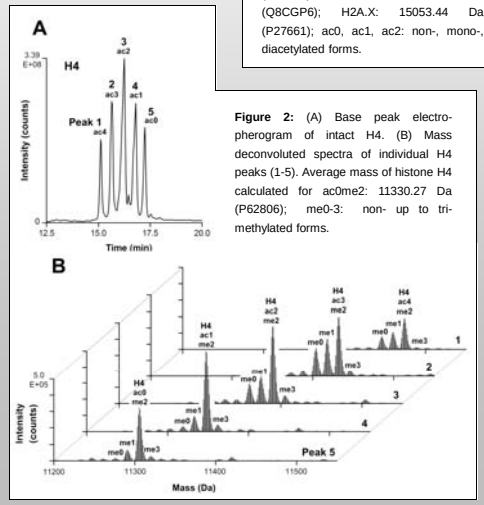

Figure 2: (A) Base peak electro-pherogram of intact H4. (B) Mass deconvoluted spectra of individual H4 peaks (1-5). Average mass of histone H4 calculated for ac0me2: 11330.27 Da (P62806); me0-3: non- up to tri-methylated forms.

Yeast Proteome

Yeast proteome: CESI-MS was used to characterize and quantify phospho-proteins and phosphopeptides present in this highly complex sample. SILAC labeling was used for relative quantification of two yeast proteomes. Protein extracts of these two strains were mixed and enzymatically digested using Lys-C. The resulting peptides were pre-separated by RP-HPLC and analyzed by CESI-MS using a neutral capillary. A total of 1244 phosphorylation sites could be assigned and 1371 phosphorylated peptides quantified. 50 phosphopeptides were found to be differently regulated relative to the non-phosphorylated protein.

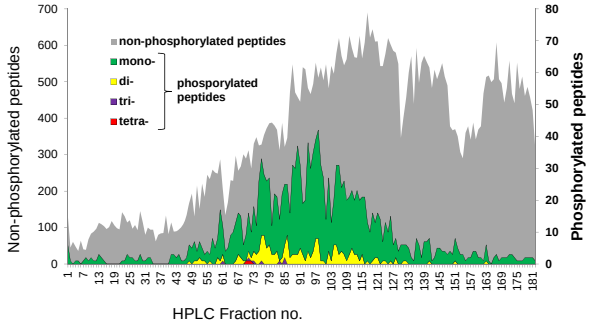

Figure 3: Phosphorylated and non-phosphorylated peptides quantified in 182 HPLC yeast fractions. Peptides were separated by RP-HPLC using a semi-preparative column (4.6 mm i.d. x 25 cm length) packed with 5 µm reversed-phase C18 material (Nucleosil), 300 Å pore size. HPLC fractions were analyzed by CESI-MS using a neutrally coated capillary.

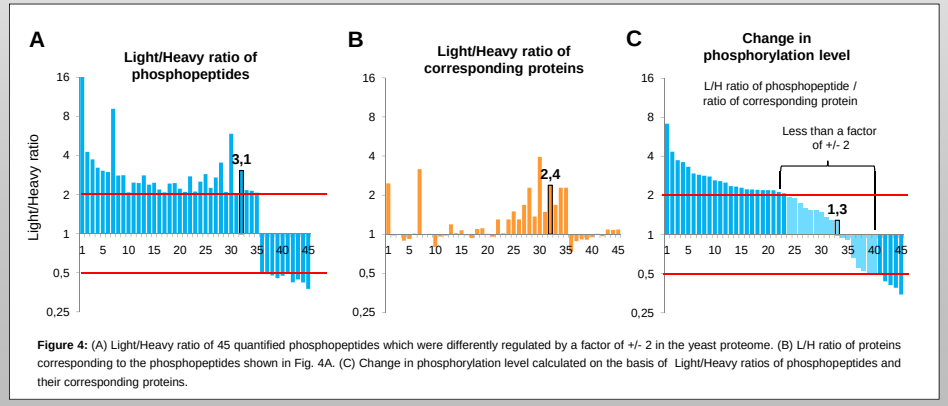

Figure 4: (A) Light/Heavy ratio of 45 quantified phosphopeptides which were differently regulated by a factor of +/- 2 in the yeast proteome. (B) L/H ratio of proteins corresponding to the phosphopeptides shown in Fig. 4A. (C) Change in phosphorylation level calculated on the basis of Light/Heavy ratios of phosphopeptides and their corresponding proteins.