Top-down Characterization of Core Histones using an Online 2D Nanocapillary LC System
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Overview
A meta-free online 2D RPLC/WCX-HILIC system was developed and coupled with Fourier transform mass spectrometry (FTMS) for comprehensive high-throughput high-sensitivity characterization of histones at the intact protein level.

Introduction
Core histones are key chromatin proteins serving as spacers to package and organize DNA into structural and manageable chromosomes. Core histones are heavily modified through lysine acetylation, lysine or arginine methylation, serine or threonine phosphorylation.

Materials and Methods

- Proteins identified using Arabidopsis thaliana (ARATH)

Partial list of identified H4 isoforms from 2D LC-MS/MS analysis.

- Methods

Norm.
Retention Accession Histone Codes P_Score Histone Number Family Theo.
Mass (Da) PTMs Matching Int. (%)
Time (min)

- Material:

H4 P62805 136.54 11383.4 11383.4 S1 acK8acK12acK20me2 3.9E-50 0.715 2D LC-MS/MS:

- Material:

(H4, H2B, H2A, H3) in the first dimension RPLC using a column developed and coupled with Fourier transform characterized of histones at the intact protein length indicates separation of the core histones in the first dimension (RPLC) and fractionation; the yellow line marks loading of collected fraction into the SPE column prior to the second dimension separation (WCX-HILIC); the red line denotes separation of one fraction in the second dimension; while the blue line displays (monoisotopic mass vs. elution time) generated from the WCX-HILIC-MS/MS of the RPLC fractions.

- Material:

In a proof-of-principle experiment using HeLa core histones, ~500 histones were identified unambiguously in a single two-dimensional (2D) LC-MS/MS analysis in 10.5 hours, increasing the gradient to 10 h in order to identify 548 isoforms for H4 alone. 25% identified histones were phosphorylated on average, and up to three phosphorylation sites per isoform were confidently identified.

- Material:

Conclusions

- Material:

A meta-free online 2D RPLC/WCX-HILIC system was developed for comprehensive high-throughput characterization of histones at the intact protein level.

- Material:

More than 500 histones were unambiguously identified (~1% FDR) in a single 24-h 2D LC-MS/MS analysis using 1.5 µg HeLa core histone mixture.

- Material:

The metal free platform enables sensitive analysis of phosphoproteins (e.g., 26% of identified histones on average were phosphorylated; 1-3 phosphorylation sites per isoform were localized).

- Material:

Acknowledgements

References

[C] Science (2010), 329:1240
[F] Nucleic Acids Research (2008), 36:275

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Figures

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Table 1. Partial list of identified H4 isoforms from 2D- LC-MS/MS analysis.

Table 2. Proteins identified using field-deployable feature extraction (FDX) database containing ten FDR (c = 15) was used for identification.

Figures

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Table 1. Partial list of identified H4 isoforms from 2D- LC-MS/MS analysis.