Characterization of the multi-acetylated sites of human histone 2A variants by Capillary Electrophoresis and Electrospray Ionization Mass Spectrometry (CESI-MS)

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ABSTRACT

Post-translational modifications (PTMs) of histones play important roles in regulating chromatin structure, gene regulation and DNA replication. Isomeric histone peptides that have similar hydrophobicity can be challenging to liquid chromatography for separation in histone characterization. Capillary electrophoresis (CE) separates analytes by hydrodynamic radius and can be used as an alternative to LC when higher separation resolution is needed. We employed CESI-MS in this work to separate histone H2A peptides containing multiple acetylation sites. Combined with data-independent acquisition (DIA), CESI-MS achieved separation of isomeric histone peptides and peptides with multi-level acetylation and identification of challenging H2A variants with only nanoliter of sample injection.

INTRODUCTION

Histones are heavily modified by dynamic PTMs which can affect the structure of chromatins and regulate DNA transcription and replication.[1,2] Histone H2A is one of the five histone proteins that is involved in function-dependent dynamic organization of chromatin. Among core histones, the H2A family exhibits the highest sequence divergence, resulting in the largest number of variants known. They often vary from each other by only a few amino acids, mostly at their C-termini. Depending on H2A variant and the biological context, H2A proteins also undergo various modifications, such as phosphorylation, acetylation and ubiquitination, which can have striking biological implications. The wide range of possible modifications coupled with the high sequence similarity makes the detailed understanding of this protein family analytically challenging.

Liquid chromatography (LC) has been the major tool for peptide separation but are challenged with separation of isomeric histone peptides with similar hydrophobicity, especially when the PTM sites are adjacent.[3] Capillary electrophoresis (CE) separates analytes by size to charge ratio and is known for its high separation efficiency. Moreover, it is possible to achieve remarkable sensitivity levels by coupling CE to MS using a sheathless interface that operates in a nanoflow regime. Hence CE can be used as an alternative strategy to LC when high sensitivity and resolution is required.

Here, histone H2A peptides containing multiple acetylation sites have been characterized using CESI as the front-end separation device and the Triple-TOF 6600+ with data independent (SWATH) acquisition for identification/quantification. In this work, we identified various acetylation sites of H2A at the peptide level. Most notably, with CESI, we were able to separate positional isomers. We achieved site confirmation with MS/MS, demonstrating that the combination of CESI and MS/MS is a powerful analytical tool for comprehensively characterizing the molecules in the histone family

METHODS

Sample preparation: Nuclei from human lymphoblastic T-cells (line CCRF-CEM) was extracted with 0.2M sulfuric acid. Histones present in the supernatant were precipitated by TCA at a final concentration of 20% for 1 hour on ice. The pellet obtained by centrifugation was washed with acidified acetone. Proteins were then re-dissolved in 0.1% (v/v) β-mercaptoethanol and separated by RP-HPLC using a C4 column (250 x 3mm; 5µm particle pore size; 30 nm pore size) and an acetonitrile gradient with the addition of 0.1% TFA (v/v). Obtained fractions were lyophilized. For enzymatic digestion, human core histones were dissolved in ammonium bicarbonate buffer (100 mM, pH 8.0) and digested overnight at 37 ºC using endoproteinase Glu-C at a ratio of 1:20. Resulting peptides were lyophilized and re-dissolved in 50 mM ammonium acetate buffer (pH 4.0) to a concentration of 1µg/µL. Prior analysis, lyophilized samples (5 µg) were reconstituted to 5 µL with 50 mM ammonium acetate at pH 4.

Instrument configuration: The CESI 8000 Plus equipped with a silica surface OptiMS cartridge (SCIEX, P/N B07367) was coupled with the TripleTOF 6600+ system through a NanoSpray III source and a CESI adapter (SCIEX, P/N B07363). 32 Karat software and Analyst TF software 1.8.1 were used to operate the CESI system with the TripleTOF 6600+ system.

Capillary electrophoresis and mass spectrometry: separation condition details can be found in reference 4. The sample was injected hydrodynamically at 5 psi for 60 sec, resulting in approximately 44 nL injected volume, which is about 220 ng of total protein content on the cartridge. The BGE (100 mM acetic acid) was freshly prepared before analysis.

MS method parameters: The histone 2A variants samples were analyzed by data dependent acquisition (DDA) and data independent acquisition (SWATH acquisition) methods. The TOF MS mass range for both acquisitions were set to 300-1750 m/z, with a cycle time of ~0.7 sec.

The SWATH acquisition method was built using 20 variable Q1 windows, and the window widths were determined based on the MS ion intensity distribution using the SWATH acquisition variable window calculator.[5]

Data processing: SCIEX OS software was used to visualize and analyze the data. ProteinPilot software was used for protein identification from DDA datasets using UNIPROT KB database from March 2021 (uniport.org) with special factors, purified histones, and human species.

RESULTS

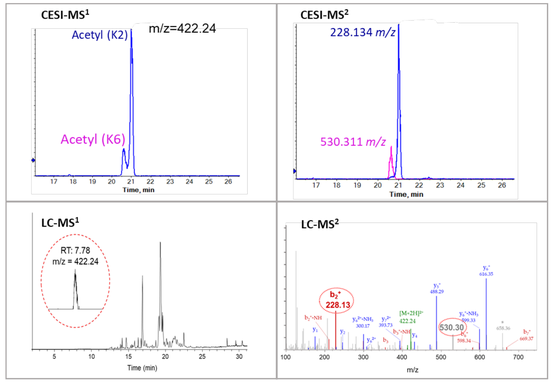

Figure 1: Characterization of acetylated peptide GKQGGKAR. Top panels show an electropherogram of the separation of mono-acetylated peptide using CESI-MS and position-specific fragments (228.134 m/z and 530.311 m/z). Bottom panels show no separation of the two mono-acetylated peptides using LC separation. Both signature fragments (228.13 m/z and 530.30 m/z) were detected in the MS/MS spectrum.

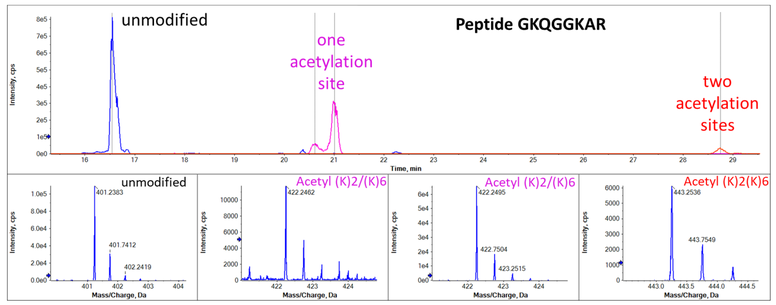

Figure 2. Acetylation sites of the GKQGGKAR peptide in H2A1 and H2A fractions. The top panel shows XICs of MS[1]. The bottom panels show precursors mass of peptide with or without modification.

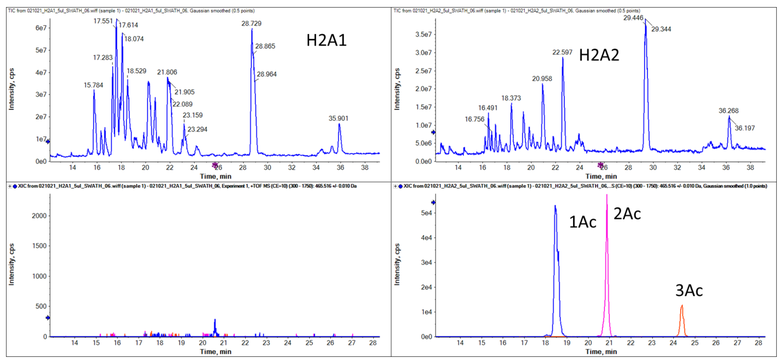

Figure 3: Separation of multi-acetylated peptides unique for H2A2 fraction. The top panels show total ion electropherogram (TIE) of fraction H2A1 and H2A2. The bottom panels show extracted electropherograms of the multi-acetyl modified peptide AGGKAGKDSGKAKTKAVSR.

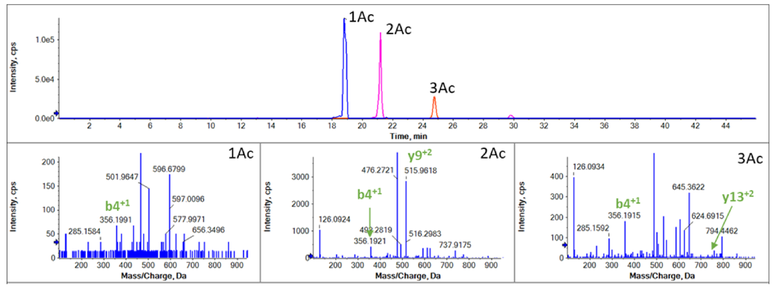

Figure 4: Characterization of the acetylation of peptide AGGKAGKDSGKAKTKAVSR in H2A2. The top panel shows separation of peptide with different acetylation (1Ac-monoacetyl, 2Ac-diacetyl, 3Ac-triacetyl). The bottom panels show the MS/MS spectrum of peptides containing K4(1Ac), K4 and K11(2Ac); K4, K7 and K11 (3Ac) to confirm the identity of the peptides.

CONCLUSIONS

- CESI-MS enabled the separation of isomeric acetylated peptides of histone, which can be difficult to be separated by LC-MS system. MS/MS spectra were used to confirm the identity of the peptides and the acetylation sites.
- Using SWATH acquisition as the MS acquisition technique ensured the identification of very challenging H2A variants with high sequence coverage
- The inherent sensitivity of CESI-MS enabled analysis from only nanoliters of sample
- Peptides with different level of acetylation (1Ac-, 2Ac- and 3Ac-) were well separated and identified by CESI-MS. The relative abundance of the acetylation variants was determined from the MS1. Even low-level acetylation can be identified using this highly sensitive platform.

REFERENCES

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4. SCIEX technical note, Characterization of the multi-acetylated sites of human histone 2A variants by Capillary Electrophoresis and Electrospray Ionization Mass Spectrometry (CESI-MS). SCIEX technical note, RUO-MKT-02-13831-A

5. the SWATH acquisition Variable Window Calculator - Excel tool. https://sciex.com/support/software-support/software-downloads

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