Journal of Proteomics 184 (2018) 34–38

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Top-down characterization of chicken core histones

Hao Wu, Kaijie Xiao, Zhixin Tian*

School of Chemical Science & Engineering, Shanghai Key Laboratory of Chemical Assessment and Sustainability, Tongji University, Shanghai 200092, China

ARTICLE INFO

Keywords:
Core histones
Chicken
Top-down proteomics
Post-translational modifications
Proteoform

ABSTRACT

Core histones and their PTMs play important roles in regulating gene transcription and other DNA-related processes. The study of core histones PTMs, their cross-talk and functional roles is not only of broad biological significance but also of wide pathological and clinical relevance. Having the strength of comprehensive proteoform identification with 100% amino acid sequence coverage and combinatorial PTMs, top-down proteomics has become the state-of-the-art analytical tool for combinatorial PTM characterization of core histones. In this study, we report our top-down characterization of chicken (Gallus gallus domesticus) core histones, which have been widely used as models for chromosome re-construction among others because of easy availability and not-so-dense PTMs. With nanoRPLC-MS/MS analysis and ProteinGoggle database search, a total of 58 proteoforms were identified for the core histone families of H4, H2B, H2A, and H3.

1. Introduction

Four distinct histone proteins form the core of eukaryotic nucleosomes; Histone H2A, H2B, H3, and H4. In a eukaryotic nucleosome, four dimer pairs with one pair from each family form the octameric core for DNA chain (about 146 base pairs) to wrap around. As chromatin proteins, histones are subject to many kinds of post-translational modifications (PTMs, such as acetylation, methylation, and phosphorylation) which play various important roles in regulating gene transcription and other DNA-related processes. Various etiologies of cancers are also related to misregulation of histone PTM patterns [1]. Certain PTMs play their roles interactively (also known as “histone code”) [2]; for example, synergistic phosphorylation and acetylation were found to be related to gene expression [3].

Thanks to the development of high-end mass spectrometers with high mass resolution and high mass measurement accuracy and powerful tandem mass spectrometry capability (various optional characteristic dissociation methods including collision-, electron-, and photon-based), mass spectrometry (MS)-based top-down proteomics has become the method-of-choice for comprehensive characterization of combinatorial PTMs of histones [4]. Using this top-down approach, Strahl et al. identified nine H2B proteoforms from yeast using LC-MS/MS (FTICR, ECD) and lysine 37 was found as a novel methylation site [5]. Using sequential ion/ion reactions and tandem mass spectrometry, Hunt et al. characterized the PTMs of histone H3.1 and identified an additional member of the H2A gene family from HeLa cells [6]. Mizzen studied H3 and H4 families of HeLa S3 cells using ESI-FTICR, and reported 13 PTMs for H3.1 and a new K20 methylation for H4 [7, 8]. An interlaboratory study of histone H4 of HeLa S3 cells was done by the consortium for top-down proteomics. About 21 credible proteoforms were reported and a new PTMs, K79 ac, was identified [9]. Stenoien et al. identified 187 core histone proteoforms from mouse brain using LC-MS/MS (Orbitrap, HCD); unexpected bromination on Y88 in H4 and other novel phosphorylation and acetylation sites in H2A were discovered [10]. Tian et al. did a comprehensive top-down characterization of HeLa core histones, and 708 proteoforms (151 from H4) were identified with 2DLC-MS/MS (Orbitrap, alternative CID/ETD) analysis [11, 12]. Pesavento et al. quantitated combinatorial modifications of human histone H4 using 2DLC-MS/MS (FTICR, ECD) and identified 42 proteoforms [13]. Brodbelt et al. characterized 288 ± 33 proteoforms of Lyophilized calf thymus histone using 193 nm Ultraviolet Photodissociation Mass Spectrometry, which shows greater sequence coverage and proficiency for characterization compared with HCD [14].

Chicken blood is easily available and its core histones are widely used in the studies of chromatin reconstitution [15], the impact of pre-modified histones [16], functional roles in transcription and replication [17], and histone conservation throughout evolution [18].

Here we report our top-down characterization of chicken blood core histones extracted from chicken erythrocytes. With RPLC-MS/MS (Orbitrap, HCD) analysis and database search using ProteinGoggle 2.0 [19–22], 58 proteoforms with combinatorial PTMs (monomethylation, dimethylation, acetylation, as well as phosphorylation) were identified.

* Corresponding author.
E-mail address: zhixintian@tongji.edu.cn (Z. Tian).

https://doi.org/10.1016/j.jprot.2018.06.007
Received 27 March 2018; Received in revised form 7 June 2018; Accepted 14 June 2018
Available online 20 June 2018

2. Experimental section

2.1. Reagents and chemicals

sodium chloride (#746398, 99.0%), sodium citrate (#W302600, 99.0%), Tris (#RDD008, 99.9%), spermidine (#85558, 99.5%), spermine (#85590, 99.0%), sucrose (#V900116, 99.0%), EDTA (#EDS, 99.0%), EGTA (#03777, 99.0%), PMSF (#78830, 99.0%), hydrochloric acid (#W530574, 32 wt% in H_2O), trichloroacetic acid (TCA, #91228, 99.5%), formic acid (FA, #F0507, 95%), and acetone (#320110, 99.5%) were brought from Sigma Aldrich (St. Louis, MO, USA). Fresh chicken (Gallus gallus domesticus) blood was obtained from local market.

Ultrapure water was produced on site by a Millipore Simplicity System (Billerica, MA, USA).

2.2. Preparation of nuclei from chicken erythrocyte

Nuclei of chicken erythrocyte were prepared according to the literature with minor modifications [23]. All steps were carried out at 4 °C. Fresh chicken blood was obtained at the sacrifice site, immediately mixed with Buffer I (15 mM sodium citrate, 150 mM NaCl, 1 mM PMSF, pH = 7.2) (*v*/v, 1:4); after sufficient shaking and mixing, the diluted blood sample was transported on ice in a cooler to the lab and stored in a − 4 °C refrigerator.

Step 1. 50 mL diluted blood sample was aliquoted and centrifuged at 3000 rpm for 5 min. The pellets were re-suspended in 40 mL Buffer I and centrifuged as described above, and this step was repeated twice.

Step 2. The pellets from Step 1 were re-suspended in 40 mL Buffer II (15 mM Tris, 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 340 mM sucrose, 2.5 mM EDTA, 0.5 mM EGTA, 1 mM PMSF, pH = 7.5) and centrifuged at 3000 rpm for 5 min. This step was repeated once.

Step 3. The pellets from Step 2 were re-suspended in 40 mL Buffer III (Buffer II with additional 0.5% NP-40) and centrifuged at 3000 rpm for 5 min. This step was repeated three times, and bright white pellets of nuclei were obtained.

Step 4. The nuclei pellets from Step 3 were re-suspended in 40 mL buffer IV (15 mM Tris, 15 mM NaCl, 60 mM KCl, 0.5 mM spermidine, 0.15 mM spermine, 340 mM sucrose, pH = 7.5) and centrifuged at 3000 rpm for 5 min. This step was repeated once, and the obtained nuclei pellets were stored at − 80 °C.

2.3. Extraction of chicken blood core histones from the nuclei

The chicken blood core histones were extracted from the nuclei prepared above with the reported protocol in the literature [24]. The nuclei pellets were re-suspended in 400 μL 0.25 N HCl (or 0.4 N H_2SO_4), incubated for 30 min on a rotator at 4 °C, and centrifuged at 16,000 *g* for 10 min. TCA (132 μL) was added to the supernatant; after incubation for 30 min, core histone precipitates were obtained by centrifugation at 16,000 *g* for 10 min. After washing with acetone twice and drying at room temperature, the core histone pellets were dissolved in 100 μL MilliQ water and stored at − 80 °C.

2.4. RPLC-MS/MS analysis of chicken blood core histones

The chicken blood core histones were separated on a Dionex Ultimate 3000 RSLC nano-HPLC system (Thermo Scientific, San Jose, CA, USA) and online analyzed on a Q Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA) with a nano-ESI source. Briefly, 3.0 μg core histones were first loaded (3.0 μL/min, 6 min) and trapped on a 5-cm long trapping column (360 μm o.d. × 200 μm i.d.) packed with Jupiter C4 particles (Phenomenex, 300 Å, 5 μm). The loading buffer was Buffer A with 4.8% ACN, 95.0% H_2O, and 0.2% FA. The trapped histone proteins were then separated on a 75-cm long analytical column (360 μm o.d. × 75 μm i.d.) packed with the same C4 particles with the trap column; Buffer B was 95.0% ACN, 4.8% H_2O, and 0.2% FA. Elution at a constant flow of 300 nL/min was conducted with the following gradient: 1–15% B for 10 min, 15–65% B for 90 min, 65–75% B for 10 min, 75–95% B for 10 min, 95% B for 10 min, 95–1% B for 10 min. MS spectra were acquired with a *m/z* range of 500–2000 and a mass resolution of 140,000 (*m/z* 200); MS/MS spectra were acquired in a Top10 data-dependent mode with HCD. The automatic gain control (AGC) target value and maximum injection time were set at 5 × 10e5 and 250 ms for MS scans, 3 × 10e6 and 250 ms for MS/MS scans. Isolation window and dynamic exclusion were set at 3 *m/z* and 20.0 s. NEC (normalized collision energy) was set at 30.0%. The temperature of the ion transfer capillary was set to 250 °C. The spray voltage was set to 2.6 kV.

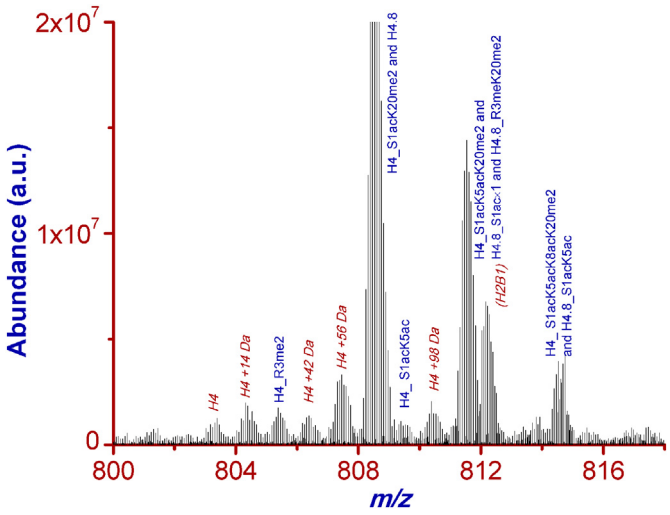

Fig. 1. Chicken H4 proteoforms identified with FDR ≤ 1% from RPLC-MS/MS analysis of the blood core histones mixture. Together shown (in italic) are also low abundant putative proteoforms identified with manual isotopic envelope fingerprinting of the precursor ions; a.u. = arbitrary unit.

2.5. Proteoform identification of chicken blood core histones

Proteoform identification of the chicken blood core histones from the RPLC-MS/MS dataset acquired above was done with ProteinGoggle 2.0. The customized database of chicken core histones with theoretical isotopic envelopes of both precursor and product ions was first built with the flat text file downloaded from Uniprot (http://www.uniprot.org/). The flat text file contains 15 proteins consisting of H4 family (H4, H4.8), H2B family (H2B1, H2B5, H2B7, H2B8), H2A family (H2A3, H2A4, H2AJ, H2AV, H2AZ), and H3 family (H32, H33); acetylation, monomethylation, dimethylation, trimethylation, phosphorylation, and crotylation in the text file were treated as dynamic PTMs. The search parameters for protein spectrum matches (PrSMs) are as follows: isotopic peak abundance cutoff (IPACO), isotopic peak *m/z* deviation (IPMD) and isotopic peak abundance deviation (IPAD) for precursor ions are 40%, 15 ppm, and 100%, respectively; IPACO, IPMD, and IPAD for product ions are 20%, 15 ppm and 50%, respectively; percentage of matching product ions (PMPs) ≥ 5%; and proteoform score ≥ 1 (i.e., number of unique product ions to distinguish a proteoform from the other putative proteoforms sharing the same precursor ion). PrSMs from target search of the forward database and decoy search of the random database were combined and rearranged in the increasing order of P score; a cutoff P score was then chosen to obtain the target PrSMs with spectrum-level FDR ≤ 1%.