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Structural Heterogeneity of Human Histone H2A.1

Khoa N. Pham[†], Yasir Mamun[†], Francisco Fernandez-Lima^{†,||,*}

[†]Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199

^{||}Biomolecular Science Institute, Florida International University, Miami, FL 33199

Abstract

Histones are highly basic chromatin proteins that tightly package and order eukaryotic DNA into nucleosomes. While the atomic structure of the nucleosomes has been determined, the three-dimensional structure of DNA-free histones remains unresolved. Here, we combine tandem non-linear and linear ion mobility spectrometry (FAIMS-TIMS) coupled to mass spectrometry in parallel with molecular modeling to study the conformational space of a DNA-free histone H2A type 1 (H2A.1). Experimental results showed the dependence of the gas-phase structures with the starting solution conditions, characterized by charge state distributions, mobility distributions, and collision-induced-unfolding (CIU) pathways. The measured H2A.1 gas-phase structures showed a high diversity of structural features ranging from compact to partially folded, and then highly elongated conformations. Molecular dynamic simulations provided candidate structures for the solution H2A.1 native conformation with folded N- and C-terminal tails, as well as gas-phase candidate structures associated with the mobility trends. Complementary CCS and dipole calculations showed that the charge distribution in the case of elongated gas-phase structures, where basic and acidic residues are mostly exposed (e.g., $z > 15+$), is sufficient to induce differences in the dipole alignment at high electric fields, in good agreement with the trends observed during the FAIMS-TIMS experiments.

Graphical Abstract

*Francisco Fernandez-Lima – Department of Chemistry and Biochemistry and Biomolecular Sciences Institute, Florida International University; fernandf@fiu.edu.

ASSOCIATED CONTENT

Supporting Information

Complete FAIMS, TIMS and MS profiles, CIU plots, TIMS-CCS_{N2} reported values, summary of all gas-phase candidate structures per charge state, instrument settings, and dipole values for the candidate structures for charge state 19+ of denatured H2A.1.

The authors declare no competing financial interest.

Histones play important roles in eukaryotic biology as they regulate the accessibility of DNA during the cellular processes¹ including transcription,² DNA replication,¹ DNA repair and recombination.³ Histones are highly basic chromatin proteins that tightly package and order eukaryotic DNA into nucleosomes, the fundamental units of chromatin.⁴ A nucleosome consists of an octameric core histone comprised of a tetramer of histones H3 and H4, as well as two dimers of histones H2A and H2B^{4–5} with approximately 147 base pairs of DNA wrapped around the histone complex.^{4–6} Nucleosomes are then connected by a DNA segment associated with a linker histone (H1 or H5) to form chromatin fibers.⁶ All members of core histones contain a solvent accessible, unstructured N-terminal tail. Among core histone members, only H2A possesses a C-terminal tail protruding out of the nucleosome core particle.⁷ These accessible N- and C-terminal tails are highly subject to post-translational modifications (PTMs),⁸ directly impacting the DNA wrapping and chromatin regulation.^{1, 8} Aberrant epigenetic regulations as a result of alterations in the patterns of histone PTMs have been associated with early events in cancer^{9–10} and other diseases.¹¹ During cellular metabolism, newly synthesized histones are transported to the nucleus by chaperones for replacement of old and/or damaged histones.⁵ Determination of structures for DNA-free histones is crucial for elucidating the function of various histones and nucleosome dynamics.^{5, 12} While the atomic structure of nucleosomes has been determined by X-ray crystallography⁴ and cryo-electron microscopy,¹³ the three-dimensional structure of DNA-free histones remains unresolved. Ion mobility spectrometry (IMS) has been established as a powerful tool for structural biology, particularly when complemented with molecular dynamics (MD) simulations.^{14–18} The capability to compare experimental ion mobilities (K) with theoretical calculations based on candidate structures makes IMS a powerful structural probe.^{15–16, 18–20} Several studies have shown the potential of non-linear IMS (i.e., field asymmetric IMS, FAIMS) to separate molecular ions based on differences in mobility (K) at low and high electric fields.^{21–24} On the other hand, linear IMS permits the measurement of the rotationally averaged CCS.^{25–26} In particular, trapped IMS (TIMS) has shown several advantages due to its small form factor, high mobility resolution, and wide mobility range to study structural features of biomolecules.^{27–32}

Here, tandem non-linear and linear IMS coupled to time-of-flight mass spectrometer (FAIMS-TIMS-TOF MS) in parallel with molecular modeling was used to study the DNA-free human histone H2A.1. The influence of the starting solvent condition (native vs. denatured) on the gas-phase structural diversity of H2A.1 is proved using FAIMS-TIMS-TOF MS and collision-induced-unfolding experiments. This is the first FAIMS-TIMS-TOF MS analysis of an intact histone that provides complementary information based on the structural dipole alignment in the FAIMS stage and CCS measurements in the TIMS stage as a function of the charge state. Molecular simulations were utilized to propose a DNA-free native solution structure and gas-phase candidate structures to interpret the trends observed during the FAIMS-TIMS-MS and CIU-TIMS-MS experiments.

EXPERIMENTAL SECTION

Materials and Reagents.

Recombinant human histone H2A.1 (accession number: P0C0S8) was purchased from EpiCypher (Durham, NC). All solvents used in this study were analytical grade or better and purchased from Fisher Scientific (Pittsburgh, PA). Low concentration Tuning Mix standard (G1969–85000) was purchased from Agilent Technologies (Santa Clara, CA) and used as received. Stock H2A.1 was prepared in ultrapure water and extensively dialyzed against 10 mM NH₄Ac buffer solution for ~ 36 h with a buffer exchange every ~ 12 h. The native and denatured H2A.1 samples were prepared by diluting concentrated histone stock to 10 μM in 10 mM aqueous NH₄Ac and 10 μM in 50 mM aqueous NH₄Ac, and in methanol:water:formic acid (v/v 50:49:1), respectively.

nESI-FAIMS-TIMS-TOF MS Instrument.

A custom-built nano electrospray ionization (nESI) FAIMS-TIMS-TOF MS instrument was utilized (Figure 1). The system consists of a “dome” FAIMS^{33–35} unit orthogonally attached to the source region of a custom built TIMS-TOF MS (Bruker Daltonics Inc., MA).^{36–37}

Ions were generated using a nESI emitter supported by a XYZ stage. Emitters were made from a quartz capillary (o.d. = 1.0 mm) using a P2000 laser puller (Sutter Instruments Co., Novato, CA). The curtain plate and nESI emitter voltages were 1.0 and 2.4 kV, respectively. Ions enter the “dome” FAIMS via a 1.5 mm circular hole on the curtain plate on the side of the external electrode. The N₂ carrier gas was supplied to the curtain orifice by a digital flowmeter (MKS Instruments) at 2 L/min; a GC filter (Agilent, RMSN-4) was used.

The “dome” FAIMS unit consists of two co-axial, nested cylindrical electrodes with an annular gap width (g_c) of 2 mm, an axial gap (g_H) of 2.4 mm at the hemispherical terminus, and an outlet aperture of 1 mm at the center.^{38–39} The FAIMS unit is coupled to the capillary inlet (0.5 mm i.d.) of the TIMS-TOF MS platform using a PEEK adapter with ~ 0.5 mm air gap for electrical insulation and discharge of excess carrier gas.³⁹ The “dome” cylindrical FAIMS system was operated by a custom control unit outputting a bi-sinusoidal waveform with a 2:1 harmonic ratio, 1 MHz frequency, and amplitude (dispersion voltage, DV) of 4 kV in negative polarity mode applied to the inner electrode (GAA Custom Electronics, LLC). The compensation voltage (CV) scan rate (S_{FAIMS}) was 1.0 V/min; the CV is expressed

as compensation field $E_C = CV/g$. To avoid potential ion activation at the FAIMS-TIMS interface, the FAIMS exit was biased at the same voltage as the TIMS-TOF MS inlet capillary.

The nESI-FAIMS-TIMS-TOF MS measurements were carried out using N_2 as a buffer gas at constant velocity defined by the pressure difference between the funnel entrance ($P_1 = 2.6$ mbar) and exit ($P_2 = 1.1$ mbar) at *ca.* 300 K.^{36–37} The TIMS radio frequency (rf) voltage was 250 Vpp at a frequency of 880 kHz. To avoid potential ion activation prior to the mobility analysis, “soft” ion transmission and trapping conditions were maintained in all experiments.^{31, 40} A voltage difference (V) between 10 to 20 V was kept between the deflector (V_{def}) and funnel entrance (V_{fun}), as well as between the funnel entrance and the TIMS analyzer (V_{ramp}). A V_{def} of -140 V, a V_{fun} of -160 V, a base voltage (V_{out}) of 60 V, and a V_{ramp} of -180 V to -20 V were used for all measurements. The TIMS scan rate (Sr_{TIMS}) was set at 1.6 V/ms.

The TIMS analyzer is comprised of a stack of segmented ring convex electrodes.⁴¹ The TIMS cell is controlled using an in-house software, written in LabVIEW (National Instruments), and synchronized with the TOF MS acquisition program. The TIMS analyzer can be operated in “transmission” mode where ions are continually pushed downstream without trapping, or in “IMS” mode where ions are trapped in the analyzer tunnel and eluted with their respective mobility by changing the electric field. The eluted ions are then transferred into the TOF MS for mass separation and detection. Reference FAIMS-TOF MS spectra were collected with TIMS operating in transmission mode (without trapping) and reference TIMS-TOF MS spectra were collected with the FAIMS unit detached. The 4-D FAIMS-TIMS-TOF MS data were acquired by summing the TIMS-MS spectra (~ 100 spectra) every 0.9 V/cm over the $E_C = -15$ to 85 V/cm range. Data were processed using Data Analysis 5.0 (Bruker Daltonics Inc., Billerica, MA) and Microcal Origin 7.0 (OriginLab, Northampton, MA).

In a TIMS device, the reduced mobility (K_0) of a given ion is directly related to the gas velocity (v_g) and can be extracted from the electric field (E) at which the ion package elutes.⁴² In practice, K_0 can be calculated from the elution voltage at which the ion package elutes (V_e) and the base voltage applied to the last electrode (V_{out}) via the expression (1):

$$K_0 = \frac{v_g}{E} \cong \frac{A}{V_e - V_{out}} \quad (1)$$

where A is a calibration constant determined using standards with known mobility. The Tuning Mix ions m/z 622.029 ($1/K_0 = 0.985$ Vs/cm²), 922.009 ($1/K_0 = 1.190$ Vs/cm²), 1221.991 ($1/K_0 = 1.382$ Vs/cm²), and 1821.952 ($1/K_0 = 1.729$ Vs/cm²) were used for calibration following the procedure described previously.^{26, 42} The K_0 of an ion is correlated to the CCS (Ω) via the Mason-Schamp equation:⁴³

$$\Omega = \frac{(18\pi)^{\frac{1}{2}}}{16} \frac{z}{(k_B T)^{\frac{1}{2}}} \left[\frac{1}{m_I} + \frac{1}{m_b} \right]^{\frac{1}{2}} \frac{1}{K_0} \frac{1}{N^*} \quad (2)$$

where z is the charge of the ion, k_B is the Boltzmann constant, T is temperature, N^* is the bath gas number density, and m_I and m_b are the masses of the molecular ion and the bath gas, respectively.^{26, 42}

CIU experiments were performed by activating the ions prior to the TIMS analyzer region with 20 V intervals by varying V_{fun} from -210 V to -30 V, while adjusting the deflector and the entrance funnel voltages accordingly.²⁹ The CIU data were analyzed using CIU Suite 2 software.⁴⁴

Classical Molecular Dynamics (CMD) Simulations.

The procedure for CMD simulations was carried out as previously described.⁴⁵ The CMD simulations were performed in explicit solvent, all-atom simulations at 310 K. The H2A.1 structure was solvated using the VMD package⁴⁶ with a TIP3P water box cutoff set to 10 Å. The initial H2A.1 structure was constructed on COOT,⁴⁷ using the high resolution (1.9 Å) X-ray structure of *Xenopus laevis* nucleosome core particle (chain C, PDB entry: 1KX5).⁴ The solvated system was electrically neutralized by adding 150 mM of NaCl randomly in the bulk water using the autoionize plugin in VMD. The CMD simulations were run using NAMD 2.14 (NVIDIA CUDA acceleration)⁴⁸ with the CHARMM36 force field.⁴⁹ The long range interactions were treated with particle mesh using the Ewald method with a 12 Å nonbonded cutoff.⁵⁰ Energy minimizations (500,000 steps) were performed using the conjugate gradient and line search algorithm, followed by heating at 1 K/ps from 0 to 310 K. After reaching 310 K, a 100 ps equilibration step with 1 fs integration time step in the NVT ensemble was performed using Langevin dynamics to maintain the temperature at 310 K. The production run of 355 ns was performed in the NPT ensemble at 1 atm and 310 K with Langevin coupling and a 2 fs integration time step. Trajectory files were saved every 10 ps for analysis.

Simulated Annealing Molecular Dynamics (SAMD) Simulations.

Gas phase SAMD simulations were performed as described previously.^{16, 51} Since the N- and C-terminal tails of the nucleosome-bound H2A.1 X-ray structure (chain C, PDB entry: 1KX5)⁴ were posed in an extended conformation, this structure may not represent the most folded conformation of H2A.1. Alternatively, an initial conformation with the smallest radius of gyration value obtained from the CMD ($R_{gyr} = 15.0$ Å, conformation I in Figure 4) was used as the starting structure for SAMD simulations. To ensure oversampling of total conformation spaces, SAMD simulations were performed for 225 cycles with the temperature ramping between T1 (300 K) and T2 (800 K): 20 ps relaxation at T1, 2 ps heating to T2, 20 ps relaxation at T2, 2 ps cooling to T1, 1 ps minimization, and 20 K increment between T1 and T2. The production run was performed in the NVT ensemble with a 1 fs integration time step. The trajectory structures were stored at every 0.5 ps for further analysis.

Steered Molecular Dynamics (SMD) Simulations.

Details on the gas phase SMD simulations can be found elsewhere.⁵² The highly elongated conformations of H2A.1 were created by pulling the center of mass of the α -carbons of residue S at position 1 (S^1) on the N-terminus at a rate of 0.25 Å/ns for 100 ns, while

anchoring residue K at position 129 (K¹²⁹) on the C-terminus. The production run was performed in the NVT ensemble with a 2 fs integration time step. The trajectory structures were stored at every 0.2 ps for further analysis.

Theoretical CCS Calculation and Cluster Analysis.

The basic concepts of theoretical CCS calculations⁵³ and cluster analysis were described previously.^{16, 54} The trajectory method (TM) in the IMoS v.1.10b^{55–58} was utilized to calculate all theoretical CCS values in N₂ gas (TMCCS_{N₂}).⁵⁹ Candidate structures are classified based on the comparison of root-mean-square deviation (RMSD) for all structures and classified into clusters.^{16, 54} The statistical processing codes⁵⁴ written in R (<https://www.r-project.org>) was used in this work. Once candidates from the cluster families were determined, charge assignment was performed by scoring the relative solvent accessibility (RSA) of basic and acidic residues in H2A.1.⁶⁰ The dipole moments of individual candidate structures were computed using YASARA software (www.yasara.org). Finally, all the structural models were displayed using PyMOL (Schrodinger, Inc.).

RESULTS AND DISCUSSION

nESI-FAIMS-TIMS-TOF MS.

Typical MS projections of H2A.1 using native (10 mM NH₄Ac and 50 mM NH₄Ac) and denaturing starting solutions (organic content) acquired using the nESI-TIMS-TOF MS configuration are shown in Figures 2 and S1, respectively. The MS of native H2A.1 exhibits a wide charge state distribution (CSD), with a shift towards high charge states as the ammonium salt content decreases and the organic content increases, which suggests “solution memory effects” during the transition from solution to the gas phase. The increase in the relative abundance of higher charge states with the addition of organic content to the starting solution has been suggested to be caused by molecular rearrangements leading to the exposure of basic residues in the solution, and during the ion formation process (e.g., changes in the droplet surface tension during the nESI process).³⁰ When compared with the MS distribution obtained using the tandem FAIMS-TIMS-MS configuration (Figures S1b, S1c, S1d, and S1e), it was observed that there is a dependence in the ion transmission using the FAIMS stage, skewing the original charge state distribution. The CCS profiles for native H2A.1 using 10 mM and 50 mM NH₄Ac (Figure S1f) using the nESI-TIMS-TOF MS configuration showed no differences; however, considering the better nESI intensity observed for the 10 mM NH₄Ac solution, this was utilized for the native FAIMS-TIMS experiments.

A comparison of CCS profiles for native and denatured H2A.1 acquired using “soft” ion injection ($V_{\text{TIMS}} = 20$ V) conditions using the nESI-TIMS-TOF MS (blue traces) and nESI-FAIMS-TIMS-TOF MS (red traces) are shown in Figures 2c and 2d for typical charged states (full profiles are shown in Figures S2b and S3b). Inspection of the native TIMS-MS CCS profiles (blue traces, Figures 2c and S2b) showed an increase in the CCS with the charge state, with multiple bands observed per charge state for most of the intermediates and higher charge states. For example, the CCS profiles of $z = 6+$ and $7+$ exhibit a narrow CCS band centered at 1600 and 1658 Å², respectively; these bands are

likely related to compact-like (C) conformations. For $z = 8+$, the CCS profile displayed a major band centered at $\sim 1734 \text{ \AA}^2$ and a minor band at $\sim 2080 \text{ \AA}^2$, indicating the coexistence of C-like structures. The CCS profiles of $z = 9+$ to $16+$ displayed many CCS bands; these correspond to partially unfolded (P) structures. The CCS profiles of $z = 17+$ to $20+$ displayed a narrow band centered at $\sim 3670, 4017, 4129$, and $4238 \text{ \AA}^2$, respectively; these correspond to elongated (E) structures. The FAIMS-TIMS-MS CCS profiles of native H2A.1 are shown in Figure 2c and S2b (red traces). The CCS profile of $z = 6+$ was not shown due to low ion transmission. For $z = 7+$ and $8+$, the CCS profiles were close to those obtained using the nESI-TIMS-MS configuration under low energy ion injection conditions. The CCS profiles for $z = 9+$ to $16+$ followed the established pattern of TIMS-MS with a transition from C- to P-like structures. Closer inspection of $z = 9+$ and $10+$ revealed differences in the CCS profiles between TIMS-MS and FAIMS-TIMS-MS (Figure 2c). For example, the CCS profile for $z = 9+$ was broader and shifted to a higher CCS when using FAIMS-TIMS-MS. Moreover, the CCS profiles for $z = 10+$ were significantly different: a narrow CCS band with a higher CCS value ($\sim 2345 \text{ \AA}^2$) was observed when using FAIMS-TIMS-MS. This indicates a subtle unfolding induced by ion heating in the FAIMS stage consistent with previous reports.^{21, 61} For higher charge states ($z = 17+$ to $20+$), no differences in the CCS profiles were observed when adding the FAIMS stage.

The CCS profiles of denatured H2A.1 acquired using TIMS-MS and FAIMS-TIMS-MS (Figures 2d and S3b) displayed differences when compared to those of native H2A.1. This further suggests that H2A.1 gas-phase structures have “solution memory effects” during the transition from solution to the gas phase, not just evidenced by changes in the CSD but also by changes in the mobility profiles (number of bands and CCS values). For example, the CCS profiles of $z = 6+, 7+$, and $8+$ exhibited narrow CCS bands at $\sim 1615, 1665$, and $1745 \text{ \AA}^2$ (Table S1), respectively, corresponding to a C-like structure. For $z = 9+$ to $13+$, the CSS profiles displayed multiple CCS bands consistent with co-existing C- and P-like structures. We also noticed more CCS features for $z = 9+$ to $11+$ of the denatured H2A.1 when compared to the native form (Table S1). Moreover, there were major differences in the CCS profiles between data obtained using the TIMS-MS and FAIMS-TIMS-MS configurations: the addition of the FAIMS stage significantly changed the CCS profiles of the P-like structures for denatured H2A.1. No differences in the CCS profiles were observed for the highest z ($17+$ to $20+$), suggesting that E-like structures are less sensitive to the ion heating of the FAIMS stage.

The compensation field profiles (E_C) of FAIMS-TOF MS (black traces) and FAIMS-TIMS-TOF MS (red traces) of native and denatured H2A.1 for typical charge states are shown in Figures 2e and 2f, respectively (all profiles are shown in and Figure S2c and S3c). No major E_C profile differences were observed between the native and denaturing starting conditions and with the inclusion of the TIMS separation. The total ion chromatogram (TIC) was comprised of ion signals ranging from -5 V/cm E_C $+60 \text{ V/cm}$, suggesting the existence of multiple structures with varying dipole alignments. The C-like structures exhibited high E_C values with a decreasing trend toward P- and E-like structures. Notably, a bimodal distribution was observed for the E-like structures (i.e., higher charge states) with the lowest E_C values.

Features in the 2-D FAIMS-TIMS palette were denoted per charge state using alphabet letters from high to low E_C values and numbers from low to high CCS values; note that these labels are charge state specific and they correspond to different types of structures. However, each denoted feature may contain different structures that are not separated based on mobility and m/z . The 2-D palettes of the lowest charge states ($z = 6+$ to $8+$) of native and denatured H2A.1 (Figures 2g, 2h, S2d, and S3d) displayed a homogenous feature denoted by A1, characteristic of a C-like structure. In addition, a feature B1 corresponding to a slightly less compact structure was observed for $z = 8+$ at lower E_C and higher CCS ranges. The 2-D palette of $z = 9+$ for native H2A.1 displayed elongated features A1 and B1 with CCS and E_C values spanning from ~ 1880 to $2225 \text{ \AA}^2$ and ~ 25 to 55 V/cm , respectively. This is consistent with co-existing ions populating C- and P-like structures. In contrast, an additional feature B2 with higher CCS ($\sim 2447 \text{ \AA}^2$), corresponding to an E-like structure was observed for the denatured H2A.1. The 2-D palettes of $z = 10+$ for native and denatured H2A.1 showed two distinct features, A1 (CCS $\sim 2345 \text{ \AA}^2$, $E_C \sim 40 \text{ V/cm}$) and B1 (CCS $\sim 2600 \text{ \AA}^2$, $E_C \sim 35 \text{ V/cm}$), suggesting co-existing structures comprised of P- and E-like conformations. In addition, feature A1 (P-like structure) was more abundant for native H2A.1, while B1 (E conformation) was more abundant for denatured H2A.1. The 2-D palettes of $z = 11+$ for native H2A.1 showed two features A1 (CCS $\sim 2491 \text{ \AA}^2$, $E_C \sim 37 \text{ V/cm}$) and A2 (CCS $\sim 2715 \text{ \AA}^2$, $E_C \sim 35 \text{ V/cm}$), corresponding to P- and E-like structures, whereas only one feature A1 (CCS $\sim 2640 \text{ \AA}^2$, $E_C \sim 33 \text{ V/cm}$) corresponding to E-like structures was observed for the denatured H2A.1. For $z = 12+$ to $14+$, two features A1 and A2 have similar E_C values but significantly different CCS values were observed for native H2A.1, suggesting P- and E- like structures. Whereas three features A1, A2, and A3 have similar E_C values but different CCS values were observed for $z=12+$ and $13+$ for denatured H2A.1. These changes in the 2D palettes with the starting solvent condition are particularly relevant since H2A.1 possesses intrinsically disordered N- and C-terminal tails, which may or may not be exposed during the transition from solution to gas phase. Moreover, an additional feature B1 at higher CCS and lower E_C values was observed for $z = 13+$ and $14+$ for denatured H2A.1, suggesting E-like structures with different dipole alignment than those observed at higher E_C (e.g., A3 of $13+$ and A2 of $14+$). For the $z = 15+$ to $17+$ of native and denatured H2A.1, the relative abundance of A1 features, corresponding to P-like structures, decreased when the charge state increased, while B1 features, at lower E_C corresponding to E-like structures with higher dipole alignment increased. For the highest charge states ($z = 18+$ to $20+$), multiple 2-D features were observed with small CCS differences (e.g., A1, A2, A3) in the ~ 4000 to $4200 \text{ \AA}^2$ CCS range; the observation of B1 features at a lower E_C suggests E-like structures with varying dipole alignments.

Impact of FAIMS stage on the CCS profiles.

The addition of the “dome” FAIMS stage led to significant decrease of the ion transmission, with $\sim 12 \%$ and $\sim 27\%$ efficiency for native and denatured studies under “soft” ion injection setting conditions, respectively (Table S2). Moreover, changes in the CCS profiles were observed with the introduction of the FAIMS stage (Figure 2), presumably due to ion heating, which was the most significant for the case of P-like structures over the $9+$ and $10+$ charge states. The ion heating in the FAIMS stage was evaluated using comparative

CIU-TIMS-MS measurements (Figure 3). The CIU fingerprints for $z = 9+$ and $10+$ are shown in Figure 3a (all profiles are shown in Figure S4).

The inspection of the CIU profiles also showed a significant influence of the starting solution – native vs. denatured H2A.1, with the largest differences among the intermediate charge states (P-like structures). For example, the CIU fingerprint of $z = 7+$ for native and denatured H2A.1 shows C- and P- like structures. At the lowest collision voltage ($\sim 10 - 50$ V), the CCS was $\sim 1650 \text{ \AA}^2$ (state 0), corresponding to C-like structures. When the collision voltage was increased from ~ 50 to 200 V, the CCS increased to $\sim 2000 \text{ \AA}^2$ (state 1), corresponding to P-like structures. At higher collision voltages ($\sim 180 - 200$ V), the relative abundance for state 0 of native H2A.1 remained high, while it decreased for the state 0 of denatured H2A.1. This result suggests that while these gas-phase structures share similar CCS values and represent C-like structures, they also present enough intramolecular differences (e.g., salt-bridges) to create different CIU patterns. In the cases of $z = 8+$, $9+$, and $10+$ the CIU profiles showed mainly three CIU states: 0 with C-like structures, 1 with P-like structures, and 2 with E-like structures, sensitive to the starting solution condition.

The comparison of the CCS profiles from CIU-TIMS and FAIMS-TIMS for the $z = 9+$ and $10+$ reveals that FAIMS heating is equivalent to ~ 240 eV collision heating at reduced pressure (~ 2 mbar), as seen in the overlay of the CIU profiles for the $z = 9+$ (26 V CIU) and $z = 10+$ (24 V CIU) shown in Figure 3. These charge states showed the largest dependence with ion heating and provided an estimate of the ion heating in the FAIMS stage.

In the case of $z = 11+$, the 0 state was observed for both native and denatured H2A.1, with lower relative abundance in the denatured form. The states 1 and 2 also showed differences; for example, multiple CCS bands from ~ 2820 to $3100 \text{ \AA}^2$ for state 2 of the denatured form. Interestingly, at the highest collision voltage ($\sim 180 - 200$ V), a decrease in CCS was observed for the denatured H2A.1. Typically, gas-phase activation results in unfolding,⁶² however, compaction of protein structures upon activation have also been reported.^{63–64} For $z = 12+$ and higher, the CIU fingerprints of both native and denatured H2A.1 were similar.

CMD Simulations of solution phase H2A.1.

Figure 4a shows the amino acid sequence and the α -helices, β -sheets, 3_{10} -helices, and the N- and C-terminal regions of H2A.1. The radius of gyration (R_{gyr}) profile along the simulation time served to identify the structural rearrangement events. For example, the snapshots (A to I) of H2A.1 conformations along the solution phase MD simulations time are shown in Figure 4b. The initial DNA bound X-ray structure of H2A.1 (A) comprises of five α -helices (α_1 to α_5), three β -sheets (β_1 to β_3), two 3_{10} -helices, together with the long and disordered N- and C-terminal tails. As shown in conformation A, these structural elements are stabilized in the nucleosome by hydrogen bonding to DNA and by interactions with α - helices from histones H3 and H4.^{4, 65} The N- and C-terminal tails of the initial X-ray structure were highly extended, leading to an E conformation with a calculated R_{gyr} value of $25.2 \text{ \AA}$. At 60 ns ($R_{\text{gyr}} = 23.9 \text{ \AA}$), the β -sheets and 3_{10} -helices were melted and the N- and C-terminal regions (α_1 and α_5) were collapsed, leading to a conformation B stabilized by salt-bridges $\text{K}^{13}/\text{E}^{56}$, $\text{K}^{15}/\text{E}^{56}$, $\text{K}^{95}/\text{E}^{92}$, $\text{K}^{118}/\text{E}^{64}$, and $\text{K}^{119}/\text{E}^{121}$. The β -sheets and 3_{10} -helices in the N- and C-terminal tails were transformed into random coil structures

consistent with previous findings for a nucleosome-free H2A-H2B heterodimer.⁶⁵ At 110 ns, the N- and C-terminal tails were gradually folded toward the α -helical core of H2A.1, leading to a partially folded conformation C ($R_{\text{gyr}} = 17 \text{ \AA}$), stabilized by salt-bridges K¹³/E⁵⁶, K⁹⁵/E⁹², K¹¹⁹/E⁶⁴, and D⁷²/K¹²⁵. At 131 ns (conformation D, $R_{\text{gyr}} = 20.3 \text{ \AA}$), the N-terminal tail was extended while the C-terminal tail was still folded and stabilized by salt-bridges K¹¹⁹/E⁶⁴, K¹²⁷/D⁹⁰, and K¹²⁹/E⁶¹. After 140 ns (conformation E, $R_{\text{gyr}} = 22.6 \text{ \AA}$), the α_1 on the N-terminal vicinity was gradually unfolded, while the C-terminal region was still folded. Subsequently, the N-terminal region was refolded with a formation of α_1 , but the C-terminal was loosely folded, leading to a conformation F ($R_{\text{gyr}} = 21.2 \text{ \AA}$) stabilized by salt-bridges R²⁰/E⁵⁶, K¹¹⁸/E¹²¹, K¹²⁹/E⁶¹, and K¹²⁹/E⁶⁴. In addition, the region in the N-terminal vicinity was further refolded, leading to a more compact structure (conformation G, $R_{\text{gyr}} = 18.0 \text{ \AA}$) stabilized by R²⁰/E⁵⁶. At 226 ns (conformation H, $R_{\text{gyr}} = 16.1 \text{ \AA}$), the α_1 was melted, leading to exposure of basic residues R³, K⁵, and R²⁰, which interact with side chains of acidic residues (E⁵⁶, E⁶⁴, and D⁷²) from α_3 . In addition, folding of the C-terminal domain is stabilized by salt-bridges K¹¹⁹/E¹²¹ and K¹²⁵/E⁶¹ between α_3 and C-terminal tail. At 310 ns, both the N- and C-terminal tails were tightly compacted (conformation I, $R_{\text{gyr}} = 15.0 \text{ \AA}$) and stabilized by electrostatic interactions between basic residues (K⁵, K⁹, K¹¹, K¹³, R¹⁷, R²⁰, R²⁹, K¹¹⁹) from N- and C-tails and acidic residues (E⁴¹, E⁵⁶, E⁶¹, E⁶⁴, D⁹⁰, E⁹²) from the α -helical core. The R_{gyr} profile did not change for an additional 45 ns, suggesting that the most stable conformation (conformation I), close to the compact conformation of H2A.1, was achieved.

Gas-phase H2A.1 structures.

Gas phase SAMD and SMD simulations generated candidate structures for the main CCS features observed during the TIMS-MS experiments (Figure S5 and Table S1). Representative candidate structures are shown as a function of the charge state and CCS range as a way to understand potential structural motifs retained from the starting native solution structure (s) (Figure 4c). For example, inspection of these candidate structures revealed major structural differences associated with the unfolding of N- and C-terminal vicinities, disruption of inter- α -helical contacts leading to exposure of charged residues, increasing the distance of N- and C-terminal domains, and extending of the N- and C-terminal tails in higher charge states ($z = 14+$).

The $z = 8+$ candidates exhibit a C-like conformation (equivalent to conformation I from CMD) with a calculated $^{\text{TM}}\text{CCS}_{\text{N}_2}$ value of $2116 \text{ \AA}^2$ comparable with a CCS value of $2100 \text{ \AA}^2$ measured by FAIMS-TIMS-MS (Table S1); this good CCS agreement suggests that H2A.1 retains most of its native solution conformation(s) during transition into the gas phase. The $z = 9+$ candidates show that the major structural changes in the gas phase are associated with an increase in distance between the N- and C-domains. The $z = 10+$ candidates reveal that the α -helical core was melted, leading to destabilization of the secondary structure and exposure of basic and acidic residues R³, K⁵, R²⁹, R³², R⁴², K⁷⁴, K⁷⁵, R⁷⁷, K⁹⁹, K¹¹⁸, K¹²⁵, and E⁶⁴. Furthermore, the α -helices were gradually transformed into β -sheets. The candidates from $11+$ to $19+$ reveal that random coil structure is the dominant structure for unfolded H2A.1. For example, inspection of candidate structures for charge state $11+$ reveal that most helical interactions were lost, followed by distancing

between the N- and C-terminal domains. Moreover, the candidate structures for $z = 12+$ and $13+$ display an increasing separation between the N- and C-terminal domains, leading to a highly E-like conformation. Inspection of candidate structures for $z = 14+$ reveals that highly extended random coil structure was the dominant motif.

Influence of the Charge Distribution on the Dipole Alignment of H2A.1.

The 2D-FAIMS-TIMS panels allowed for the investigation of the correlation between the dipole alignment, the CCS, and the charge state distribution. Experiments showed that H2A.1 gas-phase E-like structures exhibited a stronger dependence on the dipole alignment at higher z (Figures S2d and S3d). That is, extended gas-phase structures can accommodate a large variety of charge distributions leading to a wider range of dipole alignment when compared to more globular structures. The effect of the charge distribution on the dipole alignment is illustrated for the case of $z = 19+$. As shown in Figure 5a, the candidate structures representative for features A1 (CCS = 4060 Å), A2 (or B, CCS = 4112 Å), and A3 (CCS = 4165 Å) adopt E-like structures with small CCS differences. These candidates have high structural similarity as shown by the small backbone RMSD < 2.8 Å (Figure 5a). Moreover, all basic (blue sticks) and most acidic (red sticks) residues are solvent exposed. All dipole moment values for the candidate structure of the feature A2/B for $z = 19+$ were calculated in YASARA using AMBER03 force field. A total of 26 basic residues (R³, K⁵, K⁹, R¹¹, K¹², K¹⁵, R¹⁷, R²⁰, R²⁹, R³², R³⁵, K³⁶, R⁴², R⁷¹, K⁷⁴, K⁷⁵, R⁷⁷, R⁸¹, R⁸⁸, K⁹⁵, K⁹⁹, K¹¹⁸, K¹¹⁹, K¹²⁵, K¹²⁷, and K¹²⁹) were protonated, 7 out of 9 acidic residues (E⁴¹, E⁵⁶, E⁶¹, E⁶⁴, D⁷², D⁹⁰, E⁹¹, E⁹², and E¹²¹) were deprotonated, and 2 acidic residues were neutralized, leading to a combination of 36 structures with different charge distributions.

The charge distribution dependence on the dipole is shown in Figure 5b and Table S3. A closer inspection shows that the largest dipole moment value (1505 D) is observed for a structure with neutralized E⁴¹ and E⁶⁴ residues near the N-terminus, whereas the smallest dipole moment value (814 D) is obtained for a structure with neutralized E⁹² and E¹²¹ residues near the C-terminus. While we cannot exclude other potential candidate structures with similar CCS values, this example demonstrates that the charge distribution, particularly in structures with acidic and basic residues exposed, can lead to a wide distribution of the dipole alignment (broad E_C profiles). These results are in good agreement with a previous report that suggested dipole alignment under FAIMS experiments for molecular ions with dipole moments above 300 D.⁶⁶

CONCLUSIONS

In this work, we explore the gas-phase structural heterogeneity of DNA-free histones for the case of H2A.1 using tandem non-linear and linear IMS coupled to mass spectrometry in parallel with molecular simulations. The influence of the starting solvent conditions was also explored; results showed that there is a solution memory effect on the gas-phase observed structures, evidenced by characteristic CSD, mobility profiles, and CIU profiles. The investigation of gas-phase structures using TIMS-TOF MS experiments under “soft” ion injection conditions showed that H2A.1 can adopt multiple structures, ranging from compact to partially unfolded, and then to elongated structures. Under denaturing starting

conditions, a higher abundance of P- and E- like structures were observed when compared with native starting conditions. Moreover, while similar CCS profiles were observed for the C- and P- like structures from native and denatured solvent conditions, the differences in the CIU profiles suggest that there are differences in the intramolecular interactions leading to different gas-phase unfolding pathways.

The FAIMS-TIMS-MS results showed that some of the C- and E-like structures are likely stable through the FAIMS interface, while P-like structures are sensitive to the ion heating of the FAIMS interface. Comparative CIU-TIMS experiments suggested that the ion heating in FAIMS is equivalent to 240 eV collisional energy at reduced pressure (~2 mbar). In the case of E-like structures, the addition of the FAIMS interface allowed the investigation of the charge distribution and overall molecular dipole.

The CMD simulation results revealed that the solution native conformation of H2A.1 likely exists as tightly compact-like structures, where the N- and C-terminal tails are stabilized by electrostatic interactions between basic residues on the tails and acidic residues from the α -helical core. The evaluation of gas-phase candidate structures based on theoretical and experimental CCS values suggests that H2A.1 retains most of its in solution native C-like structures during the transition from solution to the gas phase. The comparison of the gas phase structures obtained using SAMD and SMD simulations with the trends observed experimentally suggests that the H2A.1 transition from C- to P-, and to E-like structures are likely associated with unfolding of the N- and C-terminal tails, melting of the α -helical core leading to exposure of basic and acidic residues, increasing distance between the N- and C-terminal domains, and extending of the N- and C-terminal tails in higher charge states ($z \geq 14+$). Theoretical dipole and CCS calculations illustrated that the charge distribution in the case of E-like structures ($z \geq 15+$) with exposed basic and acidic residues is sufficient to provide a wide range of dipoles, particularly in cases where the CCS profile shows narrow band distributions. While this work highlights the potential of tandem non-linear and linear TIMS for the study of intrinsically disordered proteins not accessible using traditional structural biology tools (NMR and X-ray), this technology can be easily translated to the study of larger proteins and protein complexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic of the online nESI-FAIMS-TIMS-TOF MS platform. The FAIMS carrier gas is denoted in blue.

Figure 2.

(a, b) Tims-MS mass spectra of H2A.1 in 10 mM NH_4Ac and denaturing starting solution, (c, d) CCS profiles acquired using Tims-MS (blue traces) and FAIMS-Tims-MS (red traces), (e, f) E_C profiles acquired in FAIMS-MS (black traces) and FAIMS-Tims-MS (red traces), (g, h) 2-D FAIMS-Tims palettes for selected charge states. The (a, c, e, g) and (b, d, f, h) plots correspond to native and denatured starting conditions, respectively. Data were acquired using “soft” Tims ion injection settings with $S_{\text{Tims}} = 1.6$ V/ms and $S_{\text{FAIMS}} = 1.0$ V/min. All charge states for the 2D FAIMS-Tims are shown in SI.

Figure 3:

(a) CIU-TIMS fingerprints, (b) overlay of CIU-TIMS (blue traces) and FAIMS-TIMS (red traces) CCS profiles for 9+ and 10+ charge states of H2A.1 from native starting conditions.

Figure 4.

Molecular modeling. (a) Amino acid sequence of H2A.1. Residues corresponding to α -helices, β -sheets, 3_{10} -helices, the N- and C-terminal regions are denoted. (b) Radius of gyration (R_{gyr}) as a function of CMD simulation time. The snapshots of native dynamics of H2A.1 are labeled from A to I. Residues corresponding to salt-bridges are indicated. (c) Depiction of potential unfolding pathways based on SAMD and SMD simulations.

Figure 5.

a) Candidate structures for features A1, A2, A3, and B for $z = 19+$ of H2A.1. b) Frequency plot of dipole moment value (μ) for all 36 structures with different charge distributions for $z = 19+$ based on candidate A2/B. The neutralized acidic residues (E and/or D) are marked with black circles.