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Structure and Dynamics in the Nucleosome Revealed by Solid-State NMR

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Abstract: Eukaryotic chromatin structure and dynamics play key roles in genomic regulation. In the current study, the secondary structure and intramolecular dynamics of human histone H4 (hH4) in the nucleosome core particle (NCP) and in a nucleosome array are determined by solid-state NMR (SSNMR). Secondary structure elements are successfully localized in the hH4 in the NCP precipitated with Mg²⁺. In particular, dynamics on nanosecond to microsecond and microsecond to millisecond timescales are elucidated, revealing diverse internal motions in the hH4 protein. Relatively higher flexibility is observed for residues participating in the regulation of chromatin mobility and DNA accessibility. Furthermore, our study reveals that hH4 in the nucleosome array adopts the same structure and show similar internal dynamics as that in the NCP assembly while exhibiting relatively restricted motions in several regions consisting of residues in the N-terminus, Loop 1 and the α 3 helix region.

DNA in eukaryotic cells is organized in nucleosomes formed by 145-147 bp DNA wrapping around the histone octamer (HO) composed of the four histone proteins, H2A, H2B, H3 and H4. The histone proteins, existing as two H2A-H2B dimers and one (H3-H4)₂ tetramer, are tightly packed and assemble the disk-shaped nucleosome core particle (NCP) structure together with DNA.^[1] Linker DNA connects NCPs to resemble a beads-on-a-string structure, which are further hierarchically packed into chromatin fibers by the binding of linker histones.^[3] The highly flexible histone tails extending outwards on the surface of the structure are subject to various posttranslational modifications (PTMs).^[4] Previous studies suggest that residues in both the flexible histone tails and the globular domains play key roles in regulating nucleosome processes.^[5] Tremendous efforts have been made in the past to characterize the atomic structures of nucleosomes, which were primarily contributed by X-ray studies^[1a, 2] and more recently by cryo-EM studies^[6]. However, these techniques still are challenging when applied to elucidating structural details and

intermolecular interactions with atomic resolution for chromatin fibers and nucleosome–protein complexes. Solid-state NMR (SSNMR) has been emerging as a powerful technique for biomolecular structure and dynamics characterization and has been successfully employed for studying various systems^[7]. In a recent study, proton-detected SSNMR experiments were performed on the NCP and NCP:LANA complex, which included ²H, ¹³C, ¹⁵N labeled H2A^[8]. This study determined the H2A secondary structure and confirmed the interface structure between the nucleosome and LANA previously determined by X-ray crystallography^[9]. The inherent plasticity of the central HO enables the NCP serving as a structural scaffold in the nucleosome compaction and facilitating DNA-templated processes.^[10] The dynamics of protein and DNA at multiple spatial and temporal scales play critical roles in regulation of chromatin functions.^[5b, 11] However, to date, quantified motional amplitudes and timescales at atomic-resolution remain poorly understood for histones in the nucleosome because the information is not directly accessible by characterization techniques such as X-ray, Cryo-EM and fluorescence spectroscopy. SSNMR is one of the premier techniques suitable for determining atomic-resolution dynamics in time scales ranging from picoseconds to seconds.^[12] In the current study, we utilized SSNMR to investigate the secondary structure and dynamics for the NCP reconstituted from the 145 bp Widom ‘601’ high affinity positioning DNA sequence^[13] and human histones, which contained ¹³C, ¹⁵N labeled human H4 (hH4). The secondary structure of hH4 in NCP was determined and, particularly, internal dynamics on the nanosecond–microsecond (ns– μ s) and microsecond–millisecond (μ s–ms) timescales was characterized in a site-specific manner. Furthermore, we elucidated the hH4 conformational and dynamic differences between the NCP and a 12-mer nucleosome array reconstituted from a 12 tandem 177 bp repeats of the high-affinity Widom ‘601’ nucleosome positioning DNA sequence (12-177-601 DNA) and human histones. This array is a well-studied and characterized in vitro model chromatin fiber. Our study provides a full map of hH4 internal motions in NCP and nucleosome array with atomic resolution and illustrates the potential contribution of histone dynamics to nucleosome biological activities.

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relatively rigid. The tail region of hH4 (S1 – D24) is not observable in all dipolar-based experiments due to significant scaling down of the relevant dipolar couplings caused by high flexibility. Several approaches are frequently employed for predicting protein secondary structure using chemical shifts and/or amino acid sequence information and provide structural information with high reliability and accuracy. Herein, we show the secondary chemical shift^[7] ($\Delta\delta$) values and the chemical shift index^[18] (CSI) as well as the TALOS-N^[19] –derived secondary structure probability (Figure 2A-2D). Overall, these structural analyses indicate that the majority of the hH4 protein including the regions K31 – R40, I50 – H75 and A83 – R92 form continuous helices. β -sheet domains consist of three short amino acid stretches, R45 – S47, R79 – V81 and R95 – Y98. As expected, the secondary structure of hH4 in the NCP extracted from our SSNMR studies agrees well with X-ray studies as shown in Figure 2E, inferring that the histones in the NCP precipitated with 20 mM Mg^{2+} is folded into the same structure as in crystals. Furthermore, near complete chemical shift assignment of backbone resonances enables characterization of the internal dynamics of hH4 in NCP as well as probing structures for nucleosome arrays and fibers as discussed in the following sections.

Figure 2. (A) Sequence alignment of hH4 protein. Residues 1-25 are not shown. (B) Secondary ^{13}C chemical shift ($\Delta\delta\text{C}$) plot, (C) CSI plot and (D) TALOS-N structure prediction for hH4 in NCP. Asterisk marks the residues having C β unassigned. (E) Schematic secondary structure representation of H4 X-ray structure in NCP (PDB: 3lzo^[2]).

SSNMR experiments were performed to characterize site-specific dynamics for hH4 in NCP. ^1H - ^{15}N and ^1H - ^{13}C dipolar line shapes were measured by using 3D dipole-chemical shift correlation experiments (DIPSHIFT)^[20], where the dipolar line shapes are extracted from the indirect recoupling dimension and the chemical sites are identified from the NCA correlations. The R-symmetry sequence^[21], R12^[4], is implemented for ^1H - ^{15}N and ^1H - ^{13}C dipolar recoupling in the 3D DIPSHIFT experiments. As shown in Figure 3A, 50 well isolated peaks are observed in the 2D NCA spectrum for hH4 in the NCP, of which the dipolar line shapes can be extracted unambiguously and simulated to obtain the dynamically averaged dipolar coupling values to determine the order parameters in the ns- μs timescale. Examples of the experimental dipolar line shape fits are shown in Figure 3B, revealing considerably variable dynamics in the ns- μs regime. The site-specific order parameters, S_{NH} ² and $S_{(\text{CH})\alpha}$ ², obtained as the ratio of experimental dipolar coupling constants to values in the rigid limit are summarized in Figure 4A and mapped onto the H4 structure in the NCP (Figure 4B and C). The C-terminal region, T96–G102, exhibits large-amplitude motions in the ns- μs timescale despite the fact that this region is buried in the interior of the NCP structure. It is interesting to note that significant motions exist in the hH4 C-terminus as it might associate with nucleosome assembly and mediate nucleosome mobility according to previous studies so that the observed enhanced dynamics may regulate or contribute to these processes^[22]. The S_{NH} and $S_{(\text{CH})\alpha}$ of residues between G28–R95 ranges from 0.8 to 1.0 and have no significant correlations with secondary structure. The dynamics shown in this timescale are generally librational motions that highly correlate with local structural features such as hydrogen bonds and spatial packing density. The absence of large difference across the majority of amino acids indicate uniformly compact packing of the residues in the well-ordered NCP core.

The μs –ms dynamics is typically observed as correlated motions in larger protein segments or domains and might mediate a variety of biological functions. Herein, we investigate

the dynamics in this timescale for hH4 in the NCP based on the cross-peak intensities in the 3D CANCO spectrum. Peak intensities in SSNMR dipolar-transfer based heteronuclear correlation spectrum such as CANCO, CONCA and NCA reflect protein internal mobility on the μs –ms timescale because such motions interfere with relaxation rates ($T_{1\rho}$, T_2^*) during CP transfer periods and chemical shift evolution. Figure 5 shows the site-resolved normalized CANCO cross-peak intensities, which are also mapped to the X-Ray structure. As illustrated in these plots, residues with scaled CANCO cross-peak intensities smaller than 0.4 primarily reside in G28–P32, G41–Y51, D85–A89 and T96–G102, indicating less restricted motions exhibited in these regions on the μs –ms timescales. The high flexibility of G28–P32 is not surprising as it is adjacent to the flexible N-terminal tail. The region G41–G48 is in close contact with the DNA superhelix location (SHL) ± 0.5 and the preceding residues L49–Y51 are adjacent to residues on the flat surface of NCP. The high mobility observed in this region might be associated with the dynamic interface of the HO with DNA and facilitate nucleosome unwrapping and DNA accessibility. T96–Y98 of H4 play roles in nucleosome mobility and sliding through direct interaction with the H2A C-terminus^[22b] and the observed fast motion in the μs –ms time window may contribute to these interactions. In addition, the majority of the $\alpha 3$ residues (D85–A89) also exhibit relatively enhanced dynamics, which is interesting to note for future studies of its biological role. The μs –ms motions are relatively restricted in the $\alpha 1$, $\alpha 2$ and L2 regions of hH4 and mostly attenuated in the E52–R55 stretch involved in a complex hydrogen bond network. The restricted mobility in these regions may critically contribute to the stability of the core structure of the nucleosome. Overall, we show that diverse motions exist in hH4 in the columnar hexagonal stacked NCP shedding new light on the potential contributions of the histone dynamics to nucleosome stability and biological processes.

Figure 3. (A) NCA spectrum of hH4 in NCP. Peak assignments are displayed and overlap peaks are marked with asterisks. (B) A subset of the experimental (dashed) ^1H - ^{13}C and ^1H - ^{15}N dipolar line shapes extracted from 3D DIPSHIFT experiments and simulated line shapes (solid). Additional fitting simulations are shown in supporting information.

Figure 4. (A) Order parameters, $S_{CHi\alpha}$ and S_{NH} , obtained by fitting 1H - $^{13}C\alpha$ and 1H - ^{15}N dipolar line shapes extracted from 3D DIPSHIFT SSNMR experiments. (B) $S_{CHi\alpha}$ mapped onto H4 structure (PDB: 3LZ0^[2]). (C) S_{NH} mapped onto H4 structure (PDB: 3LZ0^[2]). Residues of hH4 having no site-resolved order parameters due to peak overlap and the flexible tails are shown with cyan ribbon.

The success of using SSNMR to determine atomic-resolution information for the NCP illustrates that it is highly promising to employ this technique to characterize the structural and dynamic properties for nucleosome arrays and chromatin fibers. The X-ray study of a tetranucleosome indicated that the histones possess same structures as those in the NCP.^[23] To investigate the histone structures in nucleosome array, SSNMR experiments were performed for a nucleosome array consisting of 12 NCP units and were compared with those of the NCP. The sample concentration is ~300 mg/ml estimated based on the 1H spectrum. ^{13}C - ^{13}C DARR, NCA and NCO spectra of the NCP and the nucleosome array overlay remarkably well (Figure S7 and S8A), suggesting that the globular domain of hH4 in the NCP possesses the same secondary structure as in the nucleosome array. hH4 in the nucleosome array exhibits similar mobility to that of the NCP as evidenced by the consistent NCA and NCO peak linewidths with a standard deviation of only 10% across entire dataset. Detailed comparison of NCA and NCO normalized correlation peak heights are plotted in Figure S8B. The peak intensities exhibit similar trends across different structural domains of hH4 in the nucleosome array and the NCP, however, these intensities appears more uniform in the nucleosome array. Small differences are observed for peak intensities of residues residing in the N-terminal tail, L1 loop and the $\alpha 3$ helix in comparison with the rest of the globular domain of hH4 in the nucleosome array. This indicates that the flexible regions identified in the hH4 of the NCP possess attenuated μ -ms motions relative to the rest of the globular domain. In particular, the substantially increased K31 NCA peak intensity relative to other residues and the chemical shift perturbations observed for P32–K31, I26–N25 NCA peaks suggest that the N-terminus of the hH4 may be positioned somewhat differently in the nucleosome array and exhibit less mobility at the regions

adjacent to the structured segment. However, the N-terminal tail in the array retains structural flexibility as the cross-peaks corresponding to the residues 1–25 are absent in the cross-polarization-based spectra. J-coupling-based experiments performed for the NCP and the nucleosome array indicate that the hH4 tails present similar conformations and flexibility in the two systems (to be published elsewhere). It is also noted that the significantly decreased F100–G99 NCO cross-peak intensity indicates that relatively enhanced mobility occurs in this region of hH4 in the nucleosome array compared to precipitated NCP. Overall, we demonstrate that hH4 in the nucleosome array adopts the same structure as in the NCP and exhibits similar

Figure 5. (A) Normalized peak intensities extracted from 3D CANCO spectrum. (B) Normalized CANCO peak intensities mapped onto hH4 structure (PDB: 3LZ0). Error bars are derived from the RMSD of noise in the spectrum. A horizontal line is draw to guide visualization. Residues of hH4 having no site-resolved CANCO peaks due to overlap and the flexible tails are shown with cyan ribbon.

dynamics with μ s–ms motions more restricted in the N-terminal tail, L1 and $\alpha 3$ helix regions relative to the rest of the globular domain. Furthermore, the assignments of histones in NCP can be carried over to the nucleosome array system without conducting the time-consuming 3D experiments to facilitate the characterization of atomic structure, dynamics and intermolecular interactions involving nucleosome arrays.

Our SSNMR studies elucidate the structure and dynamics for the hH4 in the NCP and the nucleosome array. Remarkable spectral resolution is achieved and resonances are assigned for residues N25–G101. The secondary structure predicted with the SSNMR assignments is consistent with X-ray studies. Site-resolved dynamics in the ns– μ s and μ s–ms timescales were measured with 3D DIPSHIFT and CANCO experiments, respectively. The Order parameters derived from 1H - ^{15}N and 1H - ^{13}C dipolar line shapes reveals a highly flexible C-terminus and small-amplitude motions existing across the region between G28 and R95 of hH4 in NCP. Furthermore, the intensities of the site-resolved CANCO peaks report that μ s–ms motions exist in many regions of the hH4 in the NCP, which might facilitate biological processes of DNA and nucleosomes. The SSNMR study of the nucleosome array revealed that the structure of the hH4 is consistent with that in the NCP and the backbone dynamics remain similar with relative reduced flexibility at several regions in the μ s–ms regime. Our study elucidated detailed structure and dynamics information for the NCP and the nucleosome array and shed light on the contribution of dynamics to nucleosome stability, sliding and unwrapping as well as DNA accessibility, which pave the way to investigate structure and dynamics of

nucleosomes and chromatin fibers at the atomic level using SSNMR.

Experimental Section

Experimental Details are described in the Supporting Information.

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Keywords: NCP • nucleosome array • solid-state NMR • structure • dynamics

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Table of Contents

New insights into the structure and dynamics of nucleosome were revealed by solid-state NMR studies. Secondary structure was successfully determined for hH4 in the NCP and the nucleosome array and diverse dynamics were observed for hH4 in the nucleosome, suggesting the potential contribution of histone internal motions to the nucleosome structure stability and biological processes. The N-terminus, Loop1 and α 3 helix regions of hH4 exhibit more restricted motions relative to the rest of the globular domain in the nucleosome array comparing with those in the NCP.