J|A|C|S COMMUNICATIONS

Published on Web 10/01/2009

Characterization of the N-Terminal Tail Domain of Histone H3 in Condensed Nucleosome Arrays by Hydrogen Exchange and NMR

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The N-terminal tail domains (NTDs) of histones, which are targets of many post-translational modifications, play important roles in the regulation of higher-order structures of chromatin and in the regulation of gene functions. To understand the functional roles of NTDs, it is essential to characterize their conformations at various chromatin states. The crystal structure of the fundamental unit of chromatin, the nucleosome core particle,[1] has been determined at near-atomic resolution,[2] in which 146 base pairs (bps) of DNA are wrapped around an octameric core of histone proteins composed of two copies each of H2A, H2B, H3, and H4 (Figure 1A). However, the NTDs are not defined in this structure, suggesting that they have flexible conformations.

To study higher-order structures of chromatin, nucleosome arrays have been developed as model systems, and the structure of a highly packed tetranucleosome has been determined by X-ray crystallography at 9 Å resolution.[3] Because of the low resolution, no definitive conclusion could be reached regarding the conformation of the NTDs in this structure. Longer nucleosome arrays adopt increasingly more compact conformations as the Mg^{2+} concentration is raised (Figure 1B),[4] commencing at an extended 10-nm beads-on-a-string structure at <0.2 mM Mg^{2+}, through a folded 30-nm chromatin fiber at ~1 mM Mg^{2+}, to an insoluble highly condensed form at a high Mg^{2+} concentration (>2 mM). For example, these conformations sediment at 29S, (40S, 55S), and >300S for a 12-mer nucleosome array, respectively. The 10-nm fiber is not known to exist under physiological conditions and often serves as a reference state for describing the interactions in the higher-order structures of chromatin. The 30-nm chromatin fiber and the highly condensed nucleosome arrays were observed for native chromatin in interphase in yeast[5] and CHO cells.[6] To date, the conformation of NTDs in these arrays has been addressed only by sedimentation and chemical cross-linking studies coupled with protein engineering or mutagenesis.[7,8] Here we demonstrate that amide hydrogen exchange coupled with NMR can be used to characterize the conformational states of NTDs in highly condensed nucleosome arrays by applying the technique to the NTD of recombinant *Drosophila melanogaster* histone H3.

Protein amide protons can chemically exchange with their isotopes in D_2O ($NH + D_2O \rightarrow ND + HOD$), and the H/D exchange has been widely used to study protein folding, structure, and dynamics.[9] The exchange rate for an amide proton is determined by the side chains of its nearest neighboring residues in the protein sequence[10] and by its surroundings in the folded structure. For example, the exchange rate may be slowed if the amide proton participates in the formation of hydrogen bonds or is blocked by amino acid side chains in a folded structure. Amide H/D exchange has also been used to explore the conformation of peptides in amyloid fibers.[11] In such a study, DMSO is used to dissolve protein aggregates for NMR detection and for quenching the H/D exchange reaction.[12] To date, however, no H/D exchange studies have been performed for aggregates involving protein−DNA complexes.

In this study, nucleosome arrays were reconstituted with recombinant ^{15}N-labeled H3 and nonlabeled H2A, H2B, H4, and the "12_601_167" DNA of Dorito et al.,[7] which includes 12 equally spaced and well-positioned nucleosomes.

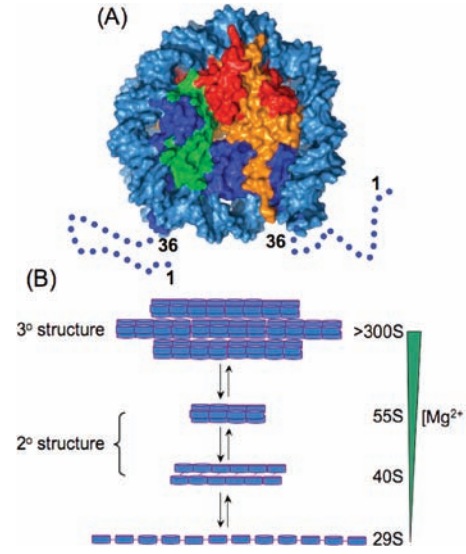

Figure 1. Nucleosome core particle structure and models of nucleosome arrays. (A) Surface representation of the nucleosome core particle structure (PDB_ID: 2PYO). Histones and DNA are color coded as follows: H2A (yellow); H2B (red); H3 (blue); H4 (green); and DNA (light blue). The dashed lines and numbers illustrate the regions of the H3 NTDs that are absent in the crystal structure. (B) Models of nucleosome arrays.[13] Disks represent nucleosomes, and lines represent linker DNAs.

The saturation of all 12 nucleosome positions was confirmed by digesting the array with restriction enzyme SacI and by running native APAGE gel, which yields the expected ladders of shorter nucleosome arrays (Figure S1). Condensed nucleosome arrays were produced by adding 10 mM Mg^{2+} to the nucleosome array solution (20 mM sodium phosphate at pH 6) and by centrifugation (Figure S2). To perform the hydrogen exchange experiment, the precipitated nucleosome arrays were then incubated for 1 h in 99.9% D_2O with the same buffer and 10 mM Mg^{2+}. To quench the H/D exchange reaction, the nucleosome arrays were precipitated again by centrifugation and by dissolving the precipitates in 95% (v/v) d_6-DMSO, 4.5% (v/v) D_2O and 0.5% d_2-dichloroacetic acid.[12] Under such conditions, the nucleosome arrays dissociate such that the

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 10.1021/ja9070078

histone proteins remain dissolved in DMSO and the DNA molecules precipitate. A $^{1}H-^{15}N$ HSQC spectrum of the solution was then taken. To measure the H/D exchange quantitatively, a reference spectrum was taken for a sample prepared similarly except that H_2O was substituted for D_2O.

Figure 2A shows the reference HSQC spectrum with peaks assigned using {^{15}N, ^{13}C}-labeled H3 and triple-resonance methods (Supporting methods). Figure 2B shows the HSQC spectrum of the sample that has exchanged in D_2O for 1 h. Clearly, some peaks in Figure 2B have weaker intensities than those in Figure 2A, whereas others retain high intensities. The proton occupancy, which is the ratio of the peak intensities in Figure 2B to those of the corresponding peaks in Figure 2A, is shown in Figure 2C.

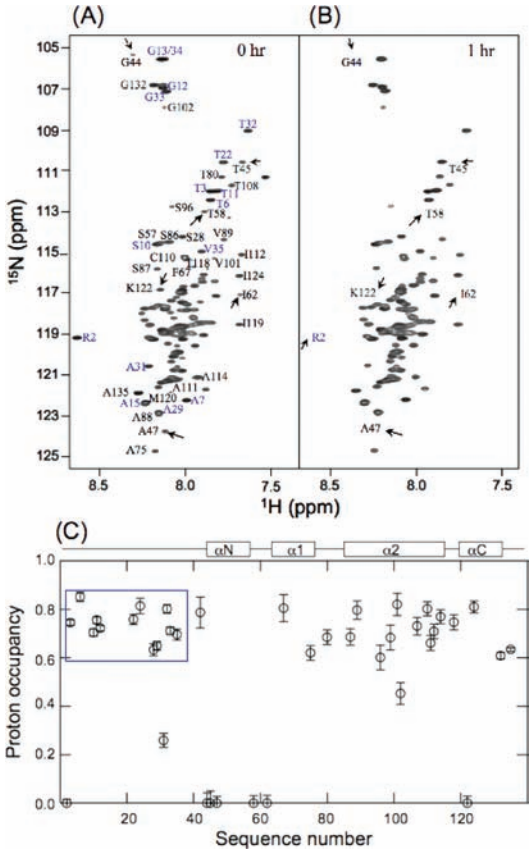

Figure 2. Hydrogen exchange of H3 in the condensed nucleosome array. $^{1}H-^{15}N$ HSQC spectra of H3 of (A) reference and (B) sample that stayed in D_2O for 1 h in the presence of 10 mM Mg^{2+} at pD_{read} 6 and 20 °C in D_2O. Arrows in (A) and (B) illustrate the amide protons whose peak intensities have decreased significantly after exchange. (C) Occupancy of amide protons in H3 after exchanging for 1 h. The spectra of (A) and (B) are measured with the same NMR parameters, and the peak intensities are normalized to the same protein concentration.

Many amide protons in the NTD of H3 show high proton occupancy, indicating that they are well protected in the condensed nucleosome arrays; amide protons of the NTD in an unfolded conformation would have exchanged in less than 0.3 min under the above exchange conditions, according to the calculated intrinsic exchange rates.[9] Indeed, the amide protons in the NTD of free H3, which forms soluble aggregates, exchange rapidly in D_2O at pD 6.0 and 20 °C in the presence of 10 mM Mg^{2+} (Figure S3). Some of the amide protons of H3 show zero proton occupancy, indicating that they have completely exchanged to deuterons. These protons are located at the N-terminus, a loop region, and the N-terminal regions of helices in the structure of the nucleosome core particle (Figure 2C), which do not form stable hydrogen bonds.

The above results show that the NTD of H3 forms stable structures in the condensed nucleosome array. They also demonstrate that amide H/D exchange coupled with NMR can be a powerful technique for studying the conformation of the NTDs of histones at the resolution of residues; this technique should be applicable to the NTDs of H2A, H2B, and H4 and those with post-translational modifications.

We note that an earlier chemical cross-linking experiment showed that the NTD of H3 engaged in internucleosome interactions in condensed nucleosome arrays.[8] However, it was not clear from that study whether the NTD has a flexible conformation that interacts only transiently with other nucleosomes or forms a stable folded structure. Our results unequivocally show that the NTD of H3 forms stable structures in the condensed nucleosome array.

Acknowledgment. We thank Drs. Carl Wu and Karolin Luger for the expression vectors of histones and DNA. This work is supported by the intramural research programs of the National Cancer Institute, the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Heart, Lung and Blood Institute, National Institutes of Health.

Supporting Information Available: Detailed experimental procedures; figures confirming saturation of 12 nucleosome positions and precipitation profiles of nucleosome array by Mg^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA9070078