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2020

Shi, X., Prasanna, C., Pervushin, K., & Nordenskiöld, L. (2020). Solid-state NMR ¹³C, ¹⁵N assignments of human histone H3 in the nucleosome core particle. *Biomolecular NMR assignments*, 14(1), 99–104. <https://doi.org/10.1007/s12104-020-09927-w>

<https://hdl.handle.net/10356/144001>

<https://doi.org/10.1007/s12104-020-09927-w>

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Solid-state NMR ^{13}C , ^{15}N assignments of human histone H3 in the nucleosome core particle

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Abstract

Nucleosome core particle (NCP), the basic unit of chromatin in eukaryotic cells, consists of ~147 bp DNA wrapped around a histone octamer (HO) formed by two H2A-H2B dimers and one (H3-H4)₄ tetramer. Histones undergo various post-translational modifications (PTMs), which regulates genomic activities in different cellular phases. High-resolution structures have been solved for many nucleosomes primarily including NCPs. However, the atomic-resolution structures of nucleosome arrays and chromatin fiber, as well as the dynamics of nucleosomes remain poorly understood. Solid-state NMR (SSNMR) is one of the premier techniques to answer these questions. In this study, we present the ¹⁵N and ¹³C chemical shifts assignments for the globular domain of human histone H3 (hH3) using multidimensional SSNMR experiments. The obtained spectra are of outstanding resolution and the assignments are nearly 100% complete for the backbone ¹³C and ¹⁵N spins of R42-G132 and ~80% when taking into account the side chains. The secondary structure derived from the chemical shifts agrees with the previously reported X-ray crystal structure. The reported chemical shifts can be carried over to future SSNMR studies of structure and dynamics of hH3 in NCPs, nucleosome array, chromatin fibers and nucleosome-protein complexes.

Keywords

Solid-state NMR; Histone; Nucleosome; Chromatin.

Biological context

About 147 bp genomic DNA in eukaryotic cells is wrapped around a histone octamer (HO) as a left-handed ~ 1.65 super helical turn, forming the basic unit of chromatin, the nucleosome core particle (NCP) (Luger et al. 1997). With linker DNA and linker histones, NCPs are further compacted to nucleosome arrays and chromatin fibers, which are the primary existing form of genomic DNA in eukaryotic cells. Histones are among the most conserved proteins in eukaryotes (Baxeavanis and Landsman 1996) and are comprised of structured globular domains and flexible N- and C- terminal regions. The globular domain is characterized by the histone-fold ($\alpha 1$ -L1- $\alpha 2$ -L2- $\alpha 3$) as determined by single crystal X-ray structure determination of the NCP (Luger et al. 1997). Amino acids in histones, both globular domain and tail regions, undergo various post translational modifications (PTMs), playing central roles in genomic activities in cell (Felsenfeld and Groudine 2003; Lawrence et al. 2016; Tessarz and Kouzarides 2014). For example, methylation of H3K79 has demonstrated to be important in telomeric silencing (Ng et al. 2002), and essential for mammalian development and heterochromatin structure (Jones et al. 2008). Acetylation of H3K56 is a key component in DNA damage response (Masumoto et al. 2005) and transcriptional regulation (Xu et al. 2005).

In the past two decades, the structures of NCPs reconstituted from various DNA and histone variants have been solved at the atomic resolution level, which have remarkably expanded our knowledge of nucleosomes and genomic activity regulation. However, the high-resolution structures of nucleosome arrays and chromatin fibers are still not fully understood. Furthermore, nucleosomes are highly dynamic at multiple spatial and temporal scales, which contribute to the regulation of biological activities of gene (Zhou et al. 2019; Shi et al. 2018). Previous studies have revealed nucleosome mobility such as DNA wrapping-unwrapping and sliding motions (Li et al. 2005; Wei et al. 2015; Zhou et al. 2019). It is challenging to quantify the dynamics of nucleosomes at various timescales in a site-specific manner. Solid-state NMR (SSNMR) is one of the premier techniques to elucidate the structures of large assembly-systems such as complexes and fibers, and can reveal site-resolved dynamic processes covering the picosecond to second timescale range (Schanda and Ernst 2016; Shi and Rienstra 2016). Therefore, SSNMR is a powerful tool for structure and dynamics studies of nucleosomes. We have previously implemented SSNMR to characterize human H4 (hH4) in a NCP and a 12-mer nucleosome array, and successfully revealed the structural features and the dynamics at nanosecond-microsecond and microsecond-

millisecond timescales (Shi et al. 2018). The study shed new light on the potential contributions of dynamics of hH4 to the nucleosome biology. To gain a more complete understanding of histones in nucleosomes, we and other research groups have been characterizing the structure and dynamics for the rest of the three histones, H3, H2A and H2B in NCPs and nucleosome arrays (Gao et al. 2013; Xiang et al. 2018; Shi et al. 2018). In the current study, we reported the ^{13}C , ^{15}N chemical shifts for human H3 (hH3), a 135-residue protein, in a NCP reconstituted from the 145 bp Widom '601' DNA positioning sequence and human histones. The chemical shift assignments will enable us to elucidate the dynamics of hH3 in nucleosomes in a site-specific fashion using SSNMR and can be carried over to future studies of the H3 histone in nucleosome arrays and chromatin fiber to investigate structure and dynamics properties (Shi et al. in progress).

Methods and experiments

Human H2A (H2A), human H2B (hH2B), and hH4 in pET-3a plasmids (gift from laboratory of Dr. Curt. A. Davey, NTU) were overexpressed as inclusion bodies in *E.coli* BL 21 (DE3) pLys S in 2X YT media by inducing with 0.4 mM IPTG at O.D 600 of 0.5. Crude histones in 7 M guanidium HCl, 20 mM sodium acetate at pH 5.2, 10 mM DTT were purified under denaturing conditions (7 M urea, 20 mM sodium acetate at pH 5.2, 1 M NaCl, 5 mM BME, 1 mM EDTA) by gel filtration using HiPrep 26/60 Sephacryl S-200 (GE Healthcare). DNA impurities bound to histones were removed by cation exchange chromatography using Resource-S column (GE Healthcare). Purity of histones was assessed by 18% SDS PAGE. Pure histones were dialyzed against water and 5 mM BME, lyophilized and stored at -80 °C.

To obtain uniformly ^{13}C , ^{15}N labeled hH3 for SSNMR studies, the hH3 in pET-3a plasmid was grown in *E.coli* BL 21 (DE3) pLys S in 2X YT media until the O.D 600 reached 0.5. The cell pellet was transferred into equal volume of M9 minimal media supplemented with 0.2% $^{13}\text{C}_6$ -glucose and 0.1% ^{15}N ammonium chloride (CIL, USA), micro nutrients and BME vitamins (Sigma-Aldrich). Following an acclimatization period of 1 hour, the cells were induced with 0.4 mM IPTG at OD 600 of 0.7. The uniformly ^{13}C , ^{15}N labeled H3 was purified following the same procedure as other histones.

hH2A, hH2B, hH4 and ^{13}C , ^{15}N - labeled H3 in equimolar ratio were refolded into HOs in 10 mM Tris·HCl at pH 7.5, 2 M NaCl, 1 mM EDTA, and 5 mM beta-mercaptoethanol at 4 °C. The HOs were purified on a HiLoad 16/600 Superdex 200 pg size-exclusion column (GE Healthcare) to remove dimers and aggregates. HOs were stored with

50% glycerol at -20 °C until usage. The quality of the HO was evaluated by the intensities of individual histones on an 18% SDS PAGE.

The pUC19 plasmid harboring eight copies of Widom '601' DNA of 145 bp flanked by EcoRV restriction sites (gift from Dr. Curt. A. Davey's laboratory, NTU) was amplified in *E.coli* DH5 α . The plasmid was isolated by alkaline lysis method and purified by RNase treatment, phenol extraction and precipitation with PEG 6000. The purified plasmid was subject to digestion with EcoRV-HF restriction enzyme (NEB). The 145 bp Widom '601' DNA was separated from the vector DNA by poly ethylene glycol 6000 mediated fractionation, followed by extraction with chloroform: isoamyl alcohol (24:1). Purity of the DNA was determined from 10% PAGE and 1.5% agarose gel electrophoresis.

The NCP was reconstituted based on the previously published method of salt gradient dialysis (Luger et al. 1999) using HO in which H3 is uniformly ^{13}C , ^{15}N labeled and 145 bp Widom '601' DNA (Lowary and Widom 1998) in a molar ratio of 0.9 HO:DNA (determined by small scale titration of HO:DNA molar ratios). The quality of NCPs was checked by evaluating both the DNA and HO on 18% SDS PAGE and 6% PAGE respectively.

The NCP pellet was prepared by precipitation with 20 mM Mg^{2+} (Berezhnoy et al. 2016). The pellet was transferred into a 3.2 mm thin wall zirconia rotor (Bruker) using ultracentrifugation at 100,000 xg for 1 hour with a rotor packing device (Giotto Biotech). ~55.2 mg of sample was packed into the rotor.

Multidimensional SSNMR experiments were performed on an 18.8 T Bruker Advance III spectrometer equipped with 3.2 mm EFree MAS probe. The actual sample temperature was controlled at 11 °C - 13 °C. The MAS spinning frequency was 15.151 kHz. ^{13}C chemical shifts were referenced with adamantane using DSS scale (downfield signal at 40.48 ppm) and ^{15}N chemical shift was indirectly calculated. 2D CC dipolar assisted rotational resonance (DARR, Takegoshi et al. 2001), CC dipolar recoupling enhanced by amplitude modulation (DREAM, Verel et al. 1998), NCA, NCO, NcaCX and 3D NCACX, NCOCX and CANCO were collected for resonance assignments. The detailed experimental parameters are summarized in Table S1 in the Supporting Information. Data was processed using nmrPipe (Delaglio et al. 1995) and analyzed with Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco). Apodization applied in the 2D CC DARR, CC DREAM data were 40 Hz Lorentzian-to-Gaussian and 60°-shifted sine-bell window functions in both dimensions. Zero-filling to 3072 and 2048 points were applied to F2 and F1

dimension, respectively. 2D NCA and NCO data were processed with 40 Hz Lorentzian-to-Gaussian and 60°-shifted sine-bell squared window functions in both dimensions, and zero-filling to 2048 and 512 points in the F2 and F1 dimension, respectively. 3D NCACX, NCOCX and CANCO data were processed with 60 Hz Lorentzian-to-Gaussian and 60°-shifted sine-bell squared window functions in all three dimensions. Zero-filling to 4096, 256 and 256 points were applied to F3, F2 and F1 dimension, respectively.

Assignments and data deposition

The SSNMR NCP sample was hydrated at ~ 65% resulting in a concentration of ~350 mg/ml, as determined from the 1D ^1H spectrum, which is in the range of chromatin concentration in vivo (50-500 mg/ml according to Bertin et al. 2007; Bohrmann et al. 1993). SSNMR spectra of the ~200 kDa complex show remarkable resolution, inferring the high conformational homogeneity and the well-ordered structure of hH3 globular domain in NCP. A two-dimensional (2D) ^{13}C - ^{13}C DARR spectrum with representative resonance assignments is shown in Fig. 1. Residues 42-132 are observed in the dipolar-based SSNMR experiments, indicating the globular domain of hH3 in the NCP is rigid. In contrast, the absence of N-terminal tail region of hH3 is attributed to its high flexibility, which can be confirmed by J-coupled SSNMR experiment according to the previous reports (Gao et al. 2013; Xiang et al. 2018) and our ongoing studies. The backbone assignments were achieved via sequential backbone walk using three-dimensional (3D) CONCA, NCOCX, and NCACX spectra. Representative sequential assignments are shown in Fig. 2, illustrating the backbone walk for residues V101 to K122. As displayed in Fig 3, complete backbone assignments are obtained unambiguously for all residues R42-G132, except for the stretch of F78-D81, and R42 and G132, which exhibit either weak or absent cross-peaks in the SSNMR spectra presumably due to less-efficient heteronuclear magnetization transfer through dipolar interactions. The absence or weak intensities of F78-D81 resonances indicate that they are more flexible in comparison with the rest of the globular domain. We attribute the high flexibility of F78-D81 to its involvement in dynamic interaction with DNA, which we will discuss elsewhere. The chemical shifts are deposited in the BioMagResBank database (accession number 28035). The secondary structure analysis was conducted with TALOS-N (Shen and Bax 2013) using the obtained chemical shifts. As shown in Fig. 4, the hH3 consist of four helices (αN $\alpha 1$, $\alpha 2$ and $\alpha 3$) connected by loops, which agree well with the X-ray crystal structure (PDB ID: 3LZ0, Vasudevan et al. 2010). The chemical shift assignments of the methyl groups agree

well with the previous solution-state NMR assignments of Ile, Leu, and Val labeled *Drosophila* H3 in a NCP with a few deviations probably arising from the differences of sample and experimental conditions (Kato et al. 2011). The nearly complete ^{13}C , ^{15}N assignments obtained for the globular domain of hH3 in the NCP will enable future NMR investigation on the structure and dynamics of histones in nucleosomes, chromatin fibers and nucleosome-protein complexes.

Acknowledgments

This work was supported by the Singapore Ministry of Education (MOE) Academic Research Fund (AcRF) Tier 2 (MOE2018-T2-1-112) and Tier 3 grants (MOE2012-T3-1-001). SSNMR experiments were conducted at the high-field NMR facility at SPMS, Nanyang Technological University. We acknowledge the NTU Institute of Structural Biology facilities for supporting this research.

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Fig. 1 1D ^{13}C - ^{13}C DARR spectrum of hH3 in the Mg^{2+} precipitated NCP, representative resonance assignments are shown. Data was collected with 100 ms DARR mixing time.

Fig. 2. Representative sequential assignment walk for hH3 in NCP. Strip plots of 3D NCACX (red), NCOCX (blue) and CANCO (black) spectra used for the sequential assignment walk are shown for residues V101-K122. The sequential connections are marked by dash lines showing inter- and intra- residue correlations.

Fig. 3. Sequential assignment graph showing carbon and nitrogen spins of hH3 R42–R132. The assigned and unassigned spins are shown in black and grey circles, respectively.

Fig. 4. A) Sequence alignment of hH3 protein. Terminal residues 1-42 and 132-135 are not shown. B) Secondary TALOS-N structure prediction for hH3 in NCP. Asterisk marks K79, of which the secondary structure is not derived from the chemical shifts due to lack of resonance assignments. C) Schematic secondary structure representation of H3 X-ray structure in NCP (PDB: 3lz0).