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A NMR study of mobility in the histone octamer

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The histone octamer from chicken erythrocytes was studied in 2 M NaCl using 500 mHz ^{1}H NMR spectroscopy. We compared the spectrum of control octamers with that of octamers isolated from trypsinized nucleosome core particles. We observe that the sharp resonances found in the spectrum of the native octamer disappear completely after trypsinization. Therefore, within the time frame of the NMR experiment, all of the mobile amino acid residues in the histone octamer are found in the well defined trypsin sensitive domains. These results indicate that there is a very clear structural demarcation between the random coil N- and C-terminal tails and the globular domains of the histones.

Nuclear magnetic resonance; Histone octamer; Chromatin; Trypsin

1. INTRODUCTION

The basic repeating structural subunit of eukaryotic chromatin is the nucleosome. The typical nucleosome consists of about 190–210 bp of DNA wrapped in two turns around the histone octamer and sealed off by a H1 linker histone. Nuclease digestion of chromatin from all eukaryotes yields a very well defined subnucleosomal particle, the nucleosome core particle, which consists of 146 bp of DNA wrapped around the histone octamer in 1.7 turns. The histone octamer is an assembly of two copies of each of the four core histones: H2A, H2B, H3, and H4 [1].

Nuclear magnetic resonance (NMR) [2–5] and protease digestion (reviewed in [6]) studies of chromatin have demonstrated that the core histones are multiple domain proteins. All four of the core histones have randomly coiled, unstructured N-terminal tails and globular, structured C-terminal domains. In addition, H2A and H3 have short C-terminal tails. Roughly 20% of the amino acid residues in the core histones are in the unstructured tail regions. Although the structure of the nucleosome core particle has been studied by neutron scattering [7,8] and X-ray crystallography [9,10], the structural and functional significance of the tail regions of the histones is still unclear.

The tails of the histones are of particular interest because they include most all of the sites of post-translational modifications. Histones are extensively and reversibly modified by acetylation, phosphorylation, methylation, ADP-ribosylation and ubiquitination [11]. For example, acetylation of the core histones, which occurs exclusively in the N-terminal tails, is strongly correlated with many DNA processing events such as transcription [12,13], DNA synthesis [14], DNA repair [15], and spermiogenesis [16]. However, structural studies of control and acetylated chromatin, especially at the core particle level, have shown no convincing structural differences ([17], summarized in [18]).

In an effort to understand more clearly the structural distinction between the N-terminal tails and the globular domains of the histones, we have studied both control and trypsinized histone octamers with 500 mHz ^{1}H NMR spectroscopy. We have found a striking structural demarcation between the trypsin sensitive tail regions and the globular domains in the histone octamer.

2. MATERIALS AND METHODS

Preparation of chicken erythrocyte core particles: Chicken nucleosome core particles were prepared as described elsewhere [19]. Briefly, chicken erythrocyte nuclei were digested with micrococcal nuclease and lysed in EDTA. The solubilized chromatin was depleted of H1 and H5 by hydroxyapatite (HAP, Calbiochem, fast-flow) column chromatography. The chromatin from the HAP column was redigested with micrococcal nuclease, and loaded onto 5–20% sucrose gradients. Fractions containing monomer core particles were pooled and dialyzed against 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 0.1 mM PMSF (dialysis buffer). When necessary the core particles were concentrated by ultrafiltration.

Trypsinization of core particles: Nucleosome core particles (2 mg/ml) were digested with 1 μg/ml trypsin (Sigma, TPCK treated) for 6.5 h at 4°C. The digestion was stopped by the addition of TLCK (Sigma) to a final concentration of 20 μg/ml. The sample was concentrated to 10 mg/ml by ultrafiltration with a Filtron ultraconcentrator (MWCO 50K) and loaded onto linear 5–20% sucrose gradients containing dialysis buffer. The gradients were centrifuged in a Beckman

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SW 28 rotor for 21 h at 26 000 rpm (4°C). Fractions containing trypsinized core particles were pooled and dialyzed against dialysis buffer.

Histone octamer preparation for the NMR studies: A HAP (Calbiochem, fast-flow) packed Omnifit glass column (2 × 15 cm) and a Pharmacia FPLC were used to fractionate histone octamers from purified core particles. Core particles were loaded onto the HAP column pre-equilibrated with 10 mM sodium phosphate, pH 7.2, and 0.1 mM PMSF. Octamers were eluted from the column with 2 M NaCl in the same buffer. Octamers were concentrated with a Filtron ultraconcentrator (MWCO 10K), and then dialyzed against 2 M NaCl, 10 mM Tris, pH 8.0 and 0.1 mM PMSF. The day before the NMR experiments the octamers were dialyzed against numerous changes of 5 mM $^2H_{11}$-Tris (Merck and Co., St. Louis, MO, USA) pH 8.0, and 2 M NaCl in ^2H_2O. The dialysis buffers also contained 0.005% DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate; Merck and Co.) which was included as an internal chemical shift standard. All dialysis steps were performed at 4°C. The concentration of octamers used in the NMR experiments was 8 mg/ml as determined by the absorbance at 277 nm [20]. The histones were analyzed on 17.5% SDS polyacrylamide gels for purity.

500 mHz nuclear magnetic resonance spectroscopy: Fourier transformed proton spectra were recorded on a Nicolet 500-mHz NMR spectrometer with quadrature detection. The carrier frequency was centered on the HOD signal, and 16 384 data points were collected over a spectral window of ± 4000 Hz with a 7-μs pulse-width and a 1.02 s acquisition time. 400 scans were accumulated with solvent suppression by presaturation over a 1-s pulse delay prior to the acquisition pulse. The samples were maintained at 4°C during the experiment. Chemical shifts were calculated from the internal standard, DSS.

3. RESULTS AND DISCUSSION

The histone octamers used in this study were analyzed on 17.5% SDS gels for the prescence of contaminating proteins and for degradation (Fig. 1). The octamers isolated by our purification procedure are virtually homogeneous. No contaminating non-histone proteins can be detected with Coomassie staining. In the control octamers, lane 1, the stoichiometry of the four core histones appears to be equal, and no degradation is visible. The octamers isolated from trypsinized core particles, lane 2, show a pattern of digestion products very similar to the pattern that has been previously published by other groups [6,21].

The 500 mHz NMR spectrum of the chicken erythrocyte histone octamer in 2 M NaCl is shown in Fig. 2A. The histone octamer complex has a combined molecular weight of 109 000 daltons. Globular proteins of this size generally have large correlation times, small T_2, and large T_1 NMR relaxation values [22]. All of these factors generally lead to line broadening in the NMR spectrum. But, the relaxation times of the mobile histone tails have previously been shown to be quite different from the relaxation times of the globular domains [23,24]. Thus, it is the mobility of the N- and C-terminal histone tails which contributes most to the sharp resonances seen in the NMR spectrum of the octamer (Fig. 2A).

The ^1H NMR spectrum of the histone octamer in 2 M NaCl at 270 and 300 mHz has been previously published

Fig. 1. 17.5% SDS-polyacrylamide gel of histones used in these studies. (Lane 1) Histones isolated from control core particles. (Lane 2) Histones from trypsinized core particles.

[2,25]. The release of the histone tails from the DNA in nucleosome core particles (between 0.3 and 0.6 M NaCl) has also been studied using 270 mHz ^1H NMR spectroscopy [2]. The NMR spectrum of the histone octamer or the nucleosome core particle in high salt are similar, due to the dissociation of the tails from the DNA. At 500 mHz, the spectrum of the histone octamer (Fig. 2A) is qualitatively similar to the spectrum at 300 mHz, although all of the resonances are much sharper and better defined. In particular, the peaks at 1.2 ppm appear as a single peak at 300 mHz, and the ring-current shifted [2] protons at 0.6 ppm in our 500 mHz spectrum are barely detectable at 300 mHz. In addition, many of the smaller peaks between 1.9 and 2.9 ppm are clearly resolved for the first time. The upfield region of the spectrum has only two peaks, from the histidines in the tails (Table I), and is not shown.

The spectrum of the histone octamer purified from trypsinized nucleosome core particles, also in 2 M NaCl, is shown in Fig. 2B. All of the sharp resonances that were seen in the control octamer spectrum are absent, with only broad lines due to the globular trypsin resistant domains still present. In fact, the predominant peak in the spectrum is the broad peak at about 0.9 ppm, which is due to the resonance of the apolar residues valine, leucine, and isoleucine. The ring current shifted protons at 0.6 ppm are still clearly visible. Both of these features are consistent with the globular, folded nature of the trypsin resistant domains.

If we assume that even short (less than 4 residue) 'tails' would be detectable in the NMR spectrum, then we can conclude from Fig. 2B that the trypsin is able to trim the histone tails down very close to the surface of

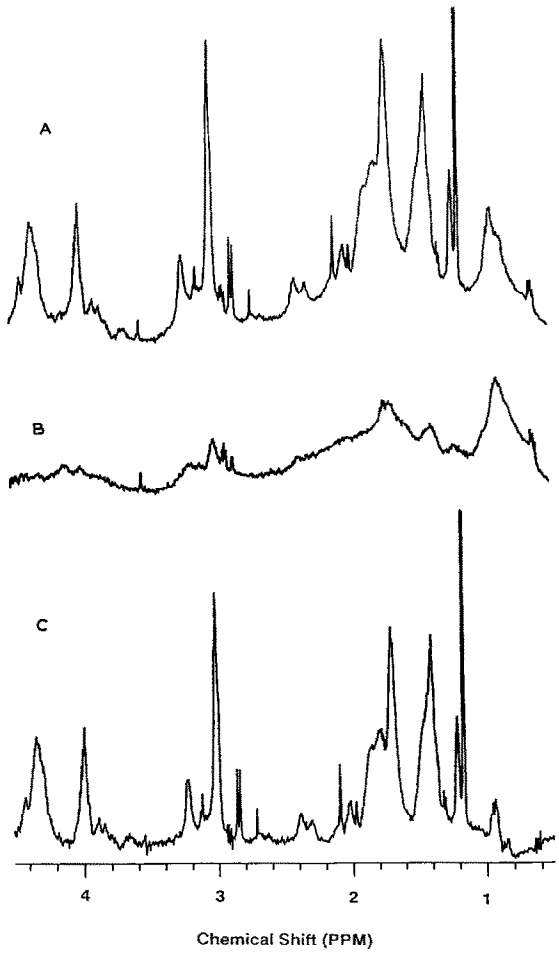

Fig. 2. 500 mHz ^{1}H NMR spectra of chicken erythrocyte histone octamers. (A) Control octamers. (B) Trypsinized octamers. (C) Difference spectrum, A minus B.

Table I

Amino acid composition and characteristic ^{1}H NMR chemical shifts of the trypsin sensitive histone tails

Amino acid residue	% of trypsin sensitive tails[a]	Characteristic ^{1}H NMR chemical shift[b]
Lysine	25%	ϵ-CH_2, 3.02 ppm
Alanine	18%	β-H, 1.39 ppm
Glycine	17%	α-H, 3.97 ppm
Arginine	11%	δ-CH_2, 3.32 ppm
Serine	6%	
Threonine	6%	γ-H, 1.23 ppm
Proline	5%	δ-H, 3.65 ppm
Glutamine	4%	δ-CH_2, 2.38 ppm
Histidine	2%	
Valine	1%	γ-CH_3, 0.97 ppm
Leucine	1%	δ-CH_3, 0.94 ppm
Isoleucine	1%	δ-CH_3, 1.19, 0.95 ppm
Glutamic acid	1%	
Aspartic acid	1%	

[a] Composition of tails based on sequences of H4(1-19), H3(1-26 and 130-135), H2A(1-11 and 119-129) and H2B(1-23) for chicken histones from [27]

[b] Random coil ^{1}H chemical shifts from [22].

the octamer. Interestingly, the DNA wrapped around the octamer in the nucleosome core particle does not seem to protect any of the random coil regions of the histones from proteolysis. This may indicate that the tails originate on a region of the octamer away from the surface where the DNA lies. This is consistent with the fact that trypsinized nucleosome core particles are thermodynamically stable, and exhibit very few biochemical differences with control core particles [21,26].

Based upon many trypsin digestion studies of chromatin, the consensus trypsin sensitive regions of the four core histones are: H2A(residues 1-11 and 119-129), H2B(1-23), H3(1-26 and 130-135) and H4(1-19) [6]. From the data presented here, we can conclude that these are the limits of the mobile tail regions as defined by NMR.

The amino acid composition of the trypsin sensitive tails is shown in Table I. In addition, the table shows the most characteristic ^{1}H NMR chemical shifts for the amino acids in the histone tails. The most striking thing about the amino acid composition of the tails is that over 70% of all the residues in the tails are lysine, alanine, glycine, and arginine. The only other abundant amino acids are threonine, serine, proline and glutamine, which account for another 20% of the tails.

The difference spectrum of the control octamers minus the trypsinized octamers is shown in Fig. 2C. The difference spectrum represents the residues which are both mobile and trypsin sensitive; and as such, is a 500 mHz spectrum of the histone tails. The most abundant residues in the tails (Table I), are represented by the predominant peaks in the difference spectrum. As expected the peaks which represent the most apolar amino acid residues, (the broad peak at 0.8-1.0 ppm and the ring current shifted groups at 0.6 ppm) are effectively subtracted out of the control spectrum.

These results show that based on NMR data there is a very clear structural distinction between the tails and the globular domains of the histone octamer: the trypsin sensitive domains contain all of the NMR mobile amino acid residues, and the globular domains have very little mobility within the time frame of the NMR experiment.

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