Analysis of the Binding of High Mobility Group Protein 17 to the Nucleosome Core Particle by $^1$H NMR Spectroscopy*

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The binding of high mobility group (HMG) protein 17 to the nucleosome core particle has been studied in D$_2$O solution using $^1$H NMR at 500 MHz. Spectra were obtained for purified HMG 17, purified nucleosome core particles, and the reconstituted HMG 17-nucleosome core particle complex at 0.1, 0.2, 0.3, and 0.4 M NaCl. Subtraction of the core particle spectra from spectra of the core particle reconstituted with HMG 17 demonstrated those regions of HMG 17 which interact with the nucleosome at different ionic strengths; the resonance peaks of interacting groups are broadened due to their restricted mobility. At 0.1 M NaCl, the mobility of all the amino acid side chains of HMG 17 was restricted, indicating complete binding of HMG 17 to the much larger nucleosome core particle. At 0.2 M NaCl most of the amino acids were free with the exception of arginine and proline which are confined to or predominant in the basic central region of HMG 17. These amino acids were completely free only at 0.4 M NaCl. We conclude that the entire HMG 17 molecule interacts with the nucleosome core particle at physiological ionic strength. The acidic COOH-terminal region of HMG 17 is released from interaction with the core histones at an NaCl concentration between 0.1 and 0.2 M and so binds weakly at physiological ionic strength. The basic central core binds more strongly to the core particle DNA, being completely released only at much higher ionic strength, between 0.3 and 0.4 M NaCl.

High mobility group (HMG)$^1$ protein 17, along with its homologous proteins HMG 14 and trout testis-specific H6, comprise a group of small basic chromosomal proteins that are thought to play a role in the structure of active and potentially active chromatin (Einck and Bustin, 1985). They are in part responsible for the nuclease sensitivity of actively transcribing regions of chromatin (reviewed in Weisbrod, 1982). Their lack of aromatic residues, limited number of hydrophobic groups, and preponderance of charged groups, 45% of HMG 17, suggest that they interact with chromatin primarily through electrostatic interactions. Their primary structure is well defined into a variably charged NH$_2$ terminus, a basic, conserved central region, and an acidic, less well conserved COOH terminus (Walker, 1982), and they are extensively and specifically posttranslationally modified (Allfrey, 1982). The genes for HMG 14 and 17 have been isolated recently and the expression of HMG 17 mRNA has been shown to be cell cycle-regulated (Bustin et al., 1987). Although these proteins have been extensively studied, their structural and functional roles in chromatin remain to be determined.

$^1$H NMR has been used to study the structure of HMG proteins in solution as well as their interaction with DNA. HMG 14 and 17 and H6 have all been found to bind free DNA through their central basic region (Abercrombie et al., 1978; Cary et al., 1980; Cary et al., 1981). $^1$H and $^13$C NMR have also been used to study the structure of histones (Lilley et al., 1975; Moss et al., 1976a, 1976b; Tancredi, 1976; Lilley et al., 1977; Nicola et al., 1978), the nucleosome core particle (Cary et al., 1978; McMurray and van Holde, 1985; Hilliard et al., 1986), and chromatin (Bradbury et al., 1973; Davies and Walker, 1974; Diaz and Walker, 1983; Walker, 1984). To help elucidate the role of HMG 17 in active chromatin we have characterized further the interaction of HMG 17 with the nucleosome core particle. We report here the analysis of HMG 17 binding to the nucleosome core particle using $^1$H NMR spectroscopy at 500 MHz. We have determined that the acidic COOH terminal region of HMG 17 binds weakly to the nucleosome core particle at physiological ionic strength, whereas the basic central region binds more strongly, being completely released only at much higher ionic strength.

EXPERIMENTAL PROCEDURES

Preparation of Samples

All procedures were carried out at 4°C unless otherwise indicated.

HMG 17

HMG 17 was isolated from fresh lamb thymus by the method of Nicolas and Goodwin (1982). The protein was dialyzed extensively...
against double distilled water, lyophilized, and dissolved to a concentration of 10 mg/ml in 99.9% D2O, 1 mM potassium phosphate buffer, pH 6.5, 10 mM NaCl (buffer A). The protein was diluted to 1 mg/ml with buffer A when taking the HMG 17 spectra alone. Protein concentration was determined by UV absorbance at 220 nm (1 mg/ml HMG 17 A20nm = 6.2).

Nucleosome Core Particles

Cell Growth—Mouse FM3A cells (approximately 6-9 × 10^6 cells/ml) were treated for 16 h with 7 mM sodium butyrate prior to harvest. The cells were lysed in isolation buffer plus 0.5% Triton X-100 (10 mM Tris-HCl, pH 6.85, 10 mM sodium butyrate, 1.5 mM MgCl2, 1.0 mM CaCl2, 0.25 M sucrose, 0.1 mM PMSF) and homogenized 15 strokes with a Dounce homogenizer. The nuclei were washed with repeated centrifugation, resuspended in isolation buffer without Triton, and stored at -70°C as a pellet (Ausio and van Holde, 1986).

Digestion of Nuclei—10 g of frozen nuclei were quickly thawed at 37°C, resuspended in 40 ml of digestion buffer (10 mM PIPES, pH 6.8, 10 mM sodium butyrate, 5 mM MgCl2, 1 mM CaCl2, 50 mM NaCl, 0.1 mM PMSF) at 37°C and A20nm was taken in NaCl-saturated urea. (1 mg/ml core DNA A20nm = 20.0.) The nuclei were preincubated at 37°C for 10 min with constant agitation and digested for 4 min with 20 units/mg DNA of micrococcal nuclease. The reaction was stopped with the addition of 1/50 volume of 100 mM EGTA on ice. The digested nuclei were centrifuged and lysed with lysis buffer (10 mM Tris-HCl, pH 6.85, 10 mM sodium butyrate, 5 mM EDTA, 0.1 mM PMSF) at 1.5 ml/h. The H1-depleted chromatin eluted as a single broad peak. The trailing two-thirds of the peak, which had a lower acetylation level, was pooled and dialyzed against 10 mM Tris-HCl, pH 6.85, 10 mM sodium butyrate, 1 mM EDTA, 0.1 mM PMSF.

Redigestion and Purification of the H1-depleted Chromatin—The concentration of chromatin was adjusted to approximately 1 mg/ml DNA and CaCl2 was added to a final concentration of 2 mM (1 mM free calcium). Chromatin was preincubated at 37°C for 15 min and digested with 40 units/mg DNA of micrococcal nuclease for 15 min with agitation. The reaction was stopped with the addition of 1/50 volume of 100 mM EGTA. The samples were concentrated using a 100,000 molecular weight cut off Filteron® ultrafiltration cell. The concentrated samples were loaded on 5-20% sucrose gradients in lysis buffer and centrifuged for 21 h at 25,000 rpm and 4°C in an SW 27 rotor. The monomer fractions were conservatively pooled through the monomer region in order to minimize dimer contamination.

Preparation of Mononucleosomes for NMR Analysis—The pooled monomer fractions were dialyzed against 1 mM potassium phosphate, pH 6.5, 10 mM NaCl. The sample was further concentrated in a ProDi-Con vacuum concentrator with an M, 60,000 cut off dialysis membrane against the same buffer. The final concentration was approximately 28 mg/ml DNA as determined by A20nm. Core particles thus prepared were dialyzed extensively against buffer A and adjusted to a concentration of 10 mg/ml with buffer A as determined by A20nm.

Reconstitution

HMG 17 (at 10 mg/ml) was added to the nucleosome core particles to a ratio of 2 HMG molecules/core particle, and the sample was mixed by gentle vortexing.

Polyacrylamide Gel Electrophoresis Analysis of Proteins and DNA

Protein was analyzed on a Laemmli/Staehelin 17.5% polyacrylamide gel (Schwab et al., 1977). Protein concentrations for HMG 17 were analyzed on 5% nondenaturing polyacrylamide gels run with 1× TBE buffer (Tris/borate/EDTA) (Sandeen et al., 1980) using HaeIII-restricted PM2 phage DNA as marker (Tatchell and van Holde, 1978).

A typical sample size used was 0.4 ml. NaCl concentration was adjusted with 5 M NaCl in D2O. Proton NMR spectra were recorded with quadrature detection on a Nicolet 500-MHz NMR spectrometer. The carrier frequency was centered on the HOD signal, and 16,384 complex data points were collected over a spectral window of ±4,000 Hz with a 7-μs pulse-width and 1.02 s acquisition time. All samples were maintained at 4°C, and the field homogeneity was adjusted to a constant water line width of 1 Hz before 400 scans, with solvent suppression, were recorded. The residual water resonance was suppressed by presaturation over a 2-s pulse delay prior to the acquisition pulse.

Methods of Analysis of NMR Data

Difference spectra were calculated with the Nicolet AS program. Chemical shifts were calculated from the residual water line, taken as 4.99 ppm. Line widths were taken as widths at half-intensity determined by least squares Lorentzian line fits. Peak assignments are for nonterminal amino acid residues in peptides as presented in Jardetzky and Roberts (1981).

RESULTS AND DISCUSSION

A 17.5% SDS-polyacrylamide gel of the proteins used in this experiment is shown in Fig. 1. Lane 1 shows the nucleosome core histones from the native core particles, and lane 2 shows the proteins after reconstitution of these core particles with HMG 17. These samples were taken after the NMR experiment, and no degradation is seen. A 5% polyacrylamide gel used in the determination of the nucleosomal DNA length is shown in Fig. 2. The DNA associated with these core particles was primarily 146 base pairs in length with most additional DNA ranging up to 160 base pairs. A nonnaturating polyacrylamide gel showed that the nucleosomes were intact before and after reconstitution with no free DNA observed (not shown).

Typical spectra of HMG 17 at 0.1, 0.2, 0.3, and 0.4 M NaCl are shown in Fig. 3. The spectra were similar to those presented by Abercrombie et al. (1978). As they observed, no difference was seen in the spectra at different salt concentrations, indicating a random coil conformation for HMG 17 when free in solution. Similar results have been found for HMG 14 (Cary et al., 1980) and H6 (Cary et al., 1981).

The spectra of the nucleosome core particles at 0.1, 0.2, 0.3, and 0.4 M NaCl are shown in Fig. 4. These spectra are similar to those presented by Cary et al. (1978) at low ionic strength. They saw little change in the core particle spectrum at different NaCl concentrations below 0.3 M. Their next highest

Fig. 1. Nucleosome core particle histones and HMG 17, 17.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Lane 1 shows the nucleosome core histones and lane 2 shows the proteins after reconstitution with two molecules HMG 17 per nucleosome core particle.
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NaCl concentration was 0.6 M at which point they noted the appearance of peaks which they interpreted to demonstrate the release of the H3 and H4 NH2-terminal tails from the nucleosome core particle. In the present study, we saw no major change in the spectrum in going from 0.1 to 0.4 M NaCl, indicating that there was no significant change in the histone-DNA interactions in the nucleosome over this salt concentration range. The spectra for the nucleosome core particles reconstituted with 2 HMG 17 molecules/core particle at 0.1, 0.2, 0.3, and 0.4 M NaCl are shown in Fig. 5. There are changes observable in this series of spectra. These changes are clearly presented in the difference spectra seen in Fig. 6.

The spectra of the nucleosome core particles reconstituted with HMG 17 minus the spectra of the core particles alone are presented in Fig. 6A. These spectra, obtained from subtracting the core spectrum alone at a given NaCl concentration from the spectrum of the cores reconstituted with HMG 17 at the same ionic strength, indicate the region(s) of the HMG 17 molecule which are freely mobile, i.e. not tightly bound to the nucleosome core particle at a given salt concentration. As regions of the HMG 17 molecule are released from the nucleosome core particle at higher ionic strengths, the amino acid side chains adopt a faster local correlation time, leading to narrower resonance line widths and thus greater peak amplitude. This increase in peak amplitude appears in these difference spectra. At 0.1 M NaCl, there is little signal above baseline showing that the entire HMG 17 molecule is bound to the core particle at this ionic strength. At 0.2 M NaCl, many more peaks are seen. The number and height of these peaks indicate that a large part of the HMG 17 molecule is freely mobile at this ionic strength. There are, however, no peaks for the arginine ΔCH2 protons (3.2 ppm) or the proline ΔCH2 protons (3.7 ppm) as are seen in the spectra of HMG 17. This indicates that the region of the HMG 17 molecule containing these amino acids, namely the central positively charged region, is still bound to the core particle at 0.2 M NaCl. At 0.3 M NaCl, the arginine and proline peaks become apparent, but these amino acids are seen as freely mobile only in the spectrum at 0.4 M NaCl. An expanded view of this region of the spectra is shown in Fig. 6B. There are also other peak amplitude increases with ionic strength at 1.7, 2.0, and 2.3 ppm. The peak at 1.7 ppm can be assigned to the β-CH3 protons of lysine and arginine. The peaks at 2.0 and 2.3 ppm can be assigned to the β- and γ-CH2 protons of proline and the γ-CH2 protons of glutamic acid and glutamine. Because
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FIG. 7. Model of the mode of binding of HMG 14/17 on the nucleosome core particle. This is our diagrammatic summary of the data presented to date regarding the binding of HMG 17 to the nucleosome core particle at low salt conditions. The order of the histones along the core DNA is from Shick et al., 1985. At or above physiological ionic strength the COOH-terminal end of HMG 17 is released from binding to histone H2A. A second HMG 17 molecule binds to the opposite face of the core particle. The length of the HMG 17 backbone is not drawn to scale and its exact path remains to be determined.

4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0 PPM

FIG. 6. Difference upfield proton spectra at 500 MHz of nucleosome core particles reconstituted with HMG 17 minus nucleosome core particles alone. a, samples were in 99.9% D2O and 1 mM potassium phosphate buffer, pH 6.5, plus 0.1, 0.2, 0.3, and 0.4 M NaCl, spectra A–D, respectively. HMG 17 concentration was 1 mg/ml; core particle concentration was 10 mg/ml. b, an expanded view of the spectra in a is shown from 3.0 to 4.0 ppm with increased scale inserts from 3.2 to 3.8 ppm.

3H NMR has been used to study the binding of HMG 17 (Abercrombie et al., 1978) as well as its homologous proteins HMG 14 (Cary et al., 1980) and H6 (Cary et al., 1981) to DNA in solution. These data all indicate that the central basic regions of these proteins bind to DNA (indicated above with italics for HMG 17). This is the same region of HMG 17 that we have identified as being tightly bound to the core particle. We conclude therefore that this region is interacting with the core particle DNA. The primary structure of this region is particularly interesting. It is the more conserved region of these molecules and contains a large number of clustered proline residues as well as an invariant 5-residue sequence Arg-Arg-Ser-Ala-Arg (residues 22–26 in HMG 17) (Walker, 1982).

In contrast to the binding studies using free DNA in which no interaction was seen between the COOH terminus of these HMG proteins and DNA, we note the complete binding of this region of HMG 17 to the nucleosome core particle at low ionic strength. Because HMG 17 (presumably the central positively charged region) cross-links to DNA (Shick et al., 1985), and the entire HMG 17 molecule is bound at 0.1 M NaCl, then the COOH-terminal region of HMG 17 could interact intramolecularly and/or with the core histone(s). HMG 17 cross-links primarily to histone H2A (Cook et al., 1986), and therefore we conclude that the COOH-terminal region interacts with histone H2A, although it may interact uniformly but to a lesser extent with the other core histones (Fig. 7). The difference spectra indicate no significant change in the mobility of the histone tails upon binding of HMG 17, indicating that HMG 17 probably binds to the globular portion of the histone(s). This is consistent with the data showing HMG 17-DNA cross-linking to the inside of the core DNA super helix (Shick et al., 1985) and the neutron scattering data suggesting that the COOH-terminal of HMG 14 is bound within the DNA super helix, releasing some portion of the DNA from interaction with the core histones (Uberbacher et al., 1982). The ends of the core DNA, however, are still bound to the core histones (Harrington et al., 1982, Sandeen et al., 1980, Yau et al., 1983). We conclude that the entire HMG 17 molecule interacts with the nucleosome core particle at physiological ionic strength. The acidic COOH-terminal region of
HMG 17 interacts weakly with core histone, possiblyglobular H2A, and is released at a NaCl concentration between 0.1 and 0.2 M. The basic NH2-terminal region binds strongly to the core particle DNA and is completely released only at much higher ionic strength, between 0.3 and 0.4 M NaCl. While HMG 17 is secured to the nucleosome core particle through its basic central region, the COOH terminus may interact in vivo with other components of active chromatin.

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