A Conformational Study of the Binding of a High Mobility Group Protein with Chromatin*

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The nature of the binding of a high mobility group protein (HMG 17) to native and H1-H5-depleted chicken erythrocyte chromatin was studied, as a function of ionic strength, using circular dichroism and thermal denaturation techniques. The circular dichroism properties of HMG 17-reconstituted whole chromatin and H1-H5-depleted chromatin demonstrated that a condensation of chromatin structure occurred upon HMG 17 binding at low ionic strength (1 mM Na phosphate, 0.25 mM EDTA, pH 7.0). Thermal denaturation profiles confirmed this change in the structure of chromatin induced by HMG 17. Thermal denaturation profiles were resolved into three-component transitions. In general, a shift in the temperature of maximum hyperchromicity found in a given transition; SD, standard deviation; *4, prepare from NaH2PO4 and adjusted to pH 7.0.

...unusually high mobility group proteins (HMG 17) are situated in the linker regions immediately adjacent to the core. The nature of the interaction of HMG 17 at higher ionic strength (50 mM NaCl, 1 mM Na phosphate, 0.25 mM EDTA, pH 7.0) with whole chromatin and H1-H5-depleted chromatin was found to be different but a decrease in [θ] values was found in both chromatins. These observations suggest that HMG 17 does not loosen chromatin structure but produces an overall stabilization and condensation of structure. The implications of these results to the currently accepted models of transcriptionally active chromatin are discussed.

Mononucleosomes released from nuclei during brief micrococcal nuclease digestion are found to be enriched in transcribing sequences (7) and are also enriched in non-histone chromosomal proteins, particularly HMG 14 and 17 (8–11). Actively transcribing genes are sensitized to DNase I in the presence of HMG 14 and 17 (12). This sensitivity can be abolished by the removal of HMG 14/17 from the nucleoli and could be re-established by the addition of HMG proteins (6, 12). Dixon and co-workers (9–13) extensively studied the digestion of trout testis nuclei and showed that HMG proteins can be major structural components of transcribing genes. The exact location of HMG proteins within the chromatin subunit is unknown.

Binding of HMG 14/17 to mononucleosomes has been previously studied (14–16). HMG 14/17 do not bind independently of each other and are found to substitute for each other (15, 16). Thermal denaturation studies have indicated that the binding sites of these proteins were in the region between 145–166 base pairs where the ends of two full turns of DNA emerge (15) after being wound around the histone core. This is consistent with the model put forward by Goodwin et al. (8).

To our knowledge, the binding of HMG 17 with high molecular weight chromatin has not been studied in detail. To probe the possible effects of HMG 17 on chromatin structure and possibly on higher ordered chromatin structure, i.e., nucleosomal interactions, studies were performed with high molecular weight chicken erythrocyte chromatin. Previously it had been shown that the DNase I sensitivity of the globin gene in chicken erythrocyte chromatin was abolished by removal of HMG 14/17 but re-established by the addition of HMG 17 (6). We report here the results of thermal denaturation and CD studies of the binding of HMG 17 to both native and H1–H5, non-histone chromosomal protein-depleted high molecular weight chicken erythrocyte chromatin (stripped chromatin). These methods are known to be sensitive indicators of histone and DNA conformations within chromatin (17). CD results indicated that HMG 17 produced a compaction of whole chromatin and stripped chromatin structure. Thermal denaturation studies indicated that HMG 17 bound to the linker regions in chromatin causing stabilization of the structure.

MATERIALS AND METHODS

Preparation of HMG 17—HMG 17 was prepared by a modified method of Walker and Johns (18). The procedure adapted is briefly discussed below. All procedures were carried out at 4 °C and all...
buffers contained 0.5 mM phenylmethylsulfonyl fluoride (Sigma, stock solution is 0.1 M in dry dioxane) as a proteolytic inhibitor. The pellet nuclei (obtained as described by Walker and Johns (18) from 2 liters of blood from freshly killed chickens were extracted twice with 400 ml of 5% perchloric acid (v/v) and centrifuged at 20,000 × g for 30 min after each extraction. The combined extract was filtered through a 1.0 μm (medium porosity) and acidified to 0.3 M HCl and precipitated by addition of 6 volumes of acetone. Approximately 2.3 g of dry protein was obtained.

The protein obtained was dissolved in approximately 60 ml of 75 mM boric acid, 0.1 mM NaCl, pH 8.8. The pH of the protein solution was adjusted to 5.0 by addition of NaOAc, and the solution was dialyzed against the same buffer overnight. This solution was concentrated, by immersing the dialysis tubing containing protein in dry Sephadex powder, to 30-ml volume. This solution was applied to a CM-Sephadex C-25 column (2.6 × 50 cm, Pharmacia), and the proteins were frac tionated using a linear salt gradient (800 ml, 0.1 mM NaCl in 75 mM borate buffer, pH 8.8, to 800 ml of 0.6 mM NaCl in the same buffer). The fractions eluting between 0.3-0.4 mM NaCl were collected and identified as HMG 17 by acetic acid urea gel electrophoresis using a marker HMG 17 kindly provided by Dr. Goodwin, Chester Beatty Research Institute, London, England. The fractions of pure HMG 17 were pooled and dialyzed against 1 mM HCl overnight and lyophilized; 6 mg of HMG 17 was obtained and stored in a desiccator under vacuum.

Chromatin-Stripping of lysine-rich histones H1 and H5 was carried out using a marker HMG 17 kindly provided by Dr. Goodwin, Chester Beatty Research Institute, London, England. The fractions of pure HMG 17 were pooled and dialyzed against 1 mM HCl and lyophilized; 6 mg of HMG 17 was obtained and stored in a desiccator under vacuum.

Removal of H1 and H5 from High Molecular Weight Whole Chromatin—Stripping of lysine-rich histones H1 and H5 was carried out using a modification of the procedure described by Thomas and Kornberg (17). A high molecular weight chromatin (A260 = 0.056) was adjusted to 0.06 M Na phosphate, pH 7.0, with an equal volume of 0.8 M NaCl, 0.1 M Na phosphate, pH 7.0. Dowex AG 50W-X2 resin, which had been converted to the Na+ form and then equilibrated with 0.4 M NaCl, 0.06 M Na phosphate, pH 7.0, was slowly added with gentle stirring (1 ml of resin/mg of DNA) at 0°C. The stirring was continued for 1 h. After the resin was allowed to settle at 0°C and the supernatant containing stripped chromatin was removed. No lysine-rich histones were detected on 13% polyacrylamide-SDS gels (22) run with 0.1 mg of total histone/gel (20). The protein/DNA ratio was 0.79 ± 0.05 g of histone/g of DNA. The stripped chromatin thus obtained was used immediately in reconstitution experiments or dialyzed down to low ionic strength (10 mM Tris-HCl, 0.25 mM EDTA, pH 7.8) and stored at 4°C. Under the above conditions, the integrity of the nucleosome repeat length is maintained (>210 ± 10 base pairs) based on slab gel electrophoresis analysis of DNA obtained from micrococcal nuclease digestion.

Reconstitution of HMG 17 with Whole Chromatin—High molecular weight whole chromatin was dialyzed against 0.35 M NaCl, 0.25 mM EDTA, 10 mM Tris-HCl, pH 7.0, and the concentration of chromatin was adjusted to A260 = 1.5 by addition of the same buffer. HMG 17 was dissolved in glass-distilled water (pH 7) to 0.75 mg/ml, and a concentration of 100 μg/ml was used for reconstitution. Varying amounts of HMG 17 and chromatin were used for reconstitution to give HMG 17/200 base pairs of DNA) molar ratios of 0, 1, and 2, and assuring the molecular weight of HMG 17 as 9247 (23, 24), Mixing was performed directly in the dialysis bags. Reconstitution was carried out using the linear gradient dialysis method of Carol (25), from 0.35 M NaCl, 0.25 mM EDTA, 10 mM Tris-HCl, pH 7.0, to 0.035 M NaCl, 10 mM Tris-HCl, 0.25 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, pH 7.0. After completion of the linear gradient dialysis, the dialysis bags were transferred into 10 mM Tris-HCl, 0.25 mM EDTA and dialysis continued for 4 h. Finally for CD and melt measurements, the solutions were dialyzed against 1.0 mM Na phosphate, 0.25 mM EDTA, pH 7.0. The solutions were centrifuged at 7000 × g for 15 min to clarify the solutions.

Reconstitution of HMG 17 with Stripped Chromatin—Reconstitution of HMG 17 with stripped chromatin was done exactly as described for whole chromatin. HMG 17 was spun down, and the solutions were dialyzed against the same buffer overnight. This solution was concentrated, by immersing the dialysis tubing containing protein in dry Sephadex powder, to 30-ml volume. This solution was applied to a CM-Sephadex C-25 column (2.6 × 50 cm, Pharmacia), and the proteins were frac tionated using a linear salt gradient (800 ml, 0.1 mM NaCl in 75 mM borate buffer, pH 8.8, to 800 ml of 0.6 mM NaCl in the same buffer). The fractions eluting between 0.3-0.4 mM NaCl were collected and identified as HMG 17 by acetic acid urea gel electrophoresis using a marker HMG 17 kindly provided by Dr. Goodwin, Chester Beatty Research Institute, London, England. The fractions of pure HMG 17 were pooled and dialyzed against 1 mM HCl overnight and lyophilized; 6 mg of HMG 17 was obtained and stored in a desiccator under vacuum.

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**Miscellaneous**—Dialysis was carried out using Spectrapor No. 3 dialysis tubing (Spectrum Medical Industries Inc., Los Angeles, CA) which had been prepared as described previously (20). Glass-distilled water and analytical grade reagents were used throughout.

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1 L. Kaplan, R. Bauer, E. Morrison, T. Langan, and G. D. Fasman, manuscript in preparation.
RESULTS AND DISCUSSION

Evaluation of HMG 17 Bound After Reconstitution

The amount of HMG 17 bound during reconstitution was determined by SDS-gel electrophoresis. The protein contents of various reconstitutes are listed in Table I. A typical gel scan (Fig. 1) shows the relative migration of HMG 17 and the histones in HMG-reconstituted whole chromatin. The relative staining intensities of HMG 17 were found to be 0.20 and 0.44 with respect to H4 for input molar ratios of 1 and 2/200 base pairs of DNA in whole chromatin. Since there are 2 molecules of H4 present for every nucleosomal repeat in chromatin, the molar ratios of HMG 17 bound after reconstitution are only 0.40 and 0.88 mol for the input molar ratios of 1 and 2/200 base pairs of DNA, respectively. Thus, approximately only 50% of added HMG 17 is bound to chromatin in our reconstitution. This is also true for the reconstitution of HMG 17 with H1-H5-stripped chromatin. This loss of material may be partly due to the adsorption of this protein on the dialysis tubing during gradient dialysis. It is known that 1 mol of HMG 17 is present for every 10 or 20 nucleosomes in whole nuclei (12). Thus, the amounts of HMG 17 bound after reconstitution are far above the ratio present in whole nuclei.

Thermal Denaturation Studies

Whole Chromatin HMG 17 Reconstitute—Whole chromatin and HMG 17-reconstituted samples were thermally denatured in solutions containing 0.25 mM EDTA, 1.0 mM Na phosphate, pH 7.00. The derivative hyperchromicity profiles of whole chromatin and HMG 17-reconstituted samples are presented in Fig. 2. The transition midpoint (T_m) as well as the relative areas of the thermal transitions (%HT) of both control and the HMG-reconstituted whole chromatin are given in Table II. Whole chromatin in the present study exhibited three well defined transitions with transition midpoints at 62, 71.6, and 83.1 °C. The first transition represents approximately 23 base pairs, the second transition 64 base pairs, and the third transition 110 base pairs.

Table I
Histone composition of reconstituted chromatin samples

| Sample            | n*       | HMG 17 | H4  | H2A | H2B | H3  | H5  | H1  |
|-------------------|----------|--------|-----|-----|-----|-----|-----|-----|
| Stripped chromatin| 1        | 0.92   | 1.18| 1.34|     |     |     |     |
|                   | 2        | 0.42   | 1.06| 1.25| 1.14|     |     |     |
|                   | 1        | 0.20   | 1.06| 1.37| 1.31| 0.80| 0.30|     |
|                   | 2        | 0.44   | 1.05| 1.47| 1.16| 0.79| 0.31|     |
| Whole chromatin   | 1        | 0.23   | 1.06| 1.27| 1.33|     |     |     |
|                   | 2        | 0.42   | 1.06| 1.25| 1.14|     |     |     |
|                   | 1        | 0.20   | 1.06| 1.37| 1.31| 0.80| 0.30|     |
|                   | 2        | 0.44   | 1.05| 1.47| 1.16| 0.79| 0.31|     |

* n = molar ratio of HMG 17/200 base pairs of DNA added in the reconstitution.

Based on SDS-polycrylamide gels stained with 0.1% Amido black. Values reported represent the staining intensity of a given histone band versus that of H4 band on the same gels. Values are reproducible within ±0.05.

Fig. 1. Electrophoretogram of histones from HMG 17-reconstituted native whole chromatin with the bound molar ratio of 0.88 HMG 17/nucleosome in SDS gels as described under "Materials and Methods."

Fig. 2. Thermal denaturation profiles (dh/dT versus T) of whole chromatin, stripped chromatin, and their reconstitution products with HMG 17 in 1 mM Na phosphate, 0.25 mM EDTA, pH 7.0. Control native whole chromatin (A), whole chromatin HMG 17 complex for bound molar ratios/200 base pairs of DNA: 0.40 (B) and 0.88 (C). Control H1-H5-stripped chromatin (D), H1-H5-stripped chromatin HMG 17 complex for bound molar ratios/200 base pairs of DNA: 0.46 (E) and 0.84 (F). Cell path length = 1 cm. Initial absorbances of chromatin samples A350 = 1 and heating rate 0.25 °C/min.
pairs and the third transition 112 base pairs of DNA (Table II). We assume that 23 base pairs of DNA melting in the first transition originate from the linker regions between the adjacent nucleosomes. The second transition, representing 64 base pairs, originates from the unstacking of weakly bound core DNA immediately adjacent to the linker region, and the third transition of 112 base pairs is from core DNA. When 0.88 mol of HMG 17/nucleosome was reconstituted with whole chromatin, the first transition almost disappeared, leaving 2% of the total hyperchromicity, and the material was distributed to the second and third transitions. A rise of about 4 °C in the Tm of the transition L was observed, whereas the Tm of transition III remained unaffected. This observation is a direct indication that HMG 17 binds to the linker regions in whole chromatin. When HMG 17 binds to the linker region, most of the DNA present in that region melts in transition III, as the %H lost in transition L is found in transition III.

**Thermal Denaturation Studies with Stripped Chromatin—** Histones H1 and H5 have been assumed to be in the inter-nucleosomal regions of whole chromatin (31). Our studies of the HMG interaction indicated the probable binding of HMG to the linker region in the presence of H1. To further investigate the role of the H1 in HMG binding, thermal denaturation studies were performed with stripped chromatin (H1–H5 and non-histone chromosomal protein-depleted).

The melting of stripped chromatin was studied in 0.25 mM EDTA, 1.0 mM Na phosphate, pH 7.0. Stripped chromatin at this ionic strength showed three well defined transitions in the denaturation profile as shown in Fig. 2 and Table II. For H1–H5-stripped chromatin, the first transition represents 50% of the hyperchromicity which corresponds to 100 base pairs of DNA, originating from 60 base pairs of the linker and 40 base pairs of the core region. Since H1–H5 are completely absent in core chromatin, linker region represents free naked DNA. The second transition, located at 70.8 °C, represents 15% of the total hyperchromicity or 30 base pairs of DNA associated with core adjacent to linker regions. The 35% of the total hyperchromicity melting in the third transition represents the remaining 70 base pairs of DNA which are strongly associated with core histones. When 0.84 mol of HMG 17/nucleosome bound to core chromatin (stripped), 8% of the hyperchromic in the transition L was lost and distributed in transitions II and III. When 0.84 mol of HMG 17/nucleosome was reconstituted, 20% of DNA melting in transition I, was distributed to the second and third transitions. The melting profile of stripped chromatin reconstituted with 0.84 mol of HMG 17/nucleosome looks different from that of native chromatin. This indicates that binding of HMG 17 is different from that of H1/H5 binding to core chromatin even though the CD values for the two samples are the same (Table III).

**Circular Dichroism Studies: Low Ionic Strength**

**CD Studies of HMG 17-reconstituted Whole Chromatin—**

The circular dichroism spectrum of whole chromatin above 250 nm is entirely due to the DNA component of chromatin, and thus can be used as a sensitive probe of DNA conformational changes of whole chromatin (17).

Whole chromatin and its HMG 17-reconstituted products were examined in solutions containing 0.25 mM EDTA, 1.0 mM Na phosphate, pH 7.0. CD spectra in the DNA region were recorded from 350 to 250 nm as shown in Fig. 3. For control whole chromatin, peak values of [θ]222.5 = 4100 ± 300 and [θ]272.5 = 3600 ± 300 deg cm²/dmol⁻¹ were found. These values are in good agreement with reported CD values in the literature (20).

For HMG-reconstituted whole chromatin, a reduction in ellipticities was always found. For bound ratios of 0.40 and 0.88 mol of HMG 17/200 base pairs of DNA, the values of [θ]222.5 = 3700 ± 300 and 3900 ± 300 deg cm²/dmol⁻¹, respectively, were obtained. The ellipticities at the shoulder at 272.5 nm were also decreased. The values of the ellipticities of whole chromatin and HMG 17-reconstituted samples are given in Table III.

**CD Studies of HMG 17-Reconstituted H1–H5-depleted Chromatin (Core Chromatin)—**

The CD spectra of control stripped chromatin and its reconstitution products with HMG 17 in 0.25 mM EDTA, 1.0 mM Na phosphate, pH 7.0, are seen in Fig. 4.

Stripped chromatin exhibits a larger maximum ellipticity in the DNA region than does the native whole chromatin due to the removal of H1–H5 histones. Complete removal of H1–H5 causes an increase of ellipticity, [θ]222.5 from 4100 to 5000 deg

![Circular dichroism spectra of native whole chromatin and its complexes with HMG 17.](image)

**Fig. 3.** Circular dichroism spectra of native whole chromatin and its complexes with HMG 17. Solution contained 1 mM Na phosphate, 0.25 mM EDTA, pH 7.0. Whole chromatin (—). Bound molar ratios of HMG 17/200 base pairs of DNA: 0.4 (-----) and 0.88 (-----). Cell path length 1 cm; A₂₅₈ of chromatin =1.1; temperature 23 °C.

**Table II**

| Sample       | HMG 17 | Input \[mol\] | Bound \[mol\] | Tm \[^{°C}\] | %Hr | Tm \[^{°C}\] | %Hr | Tm \[^{°C}\] | %Hr |
|--------------|--------|---------------|---------------|-------------|-----|-------------|-----|-------------|-----|
| Whole chromatin | 1      | 0.40          | 66.0          | 2           | 73.3| 30          | 83.3| 2           | 72.1| 28          | 82.8| 42          |
| Stripped chromatin | 1      | 0.46          | 58.4          | 42          | 71.3| 20          | 81.9| 38          |     |             |     |             |
|               | 2      | 0.84          | 61.4          | 30          | 72.1| 28          | 82.8| 42          |     |             |     |             |

\[^{a}\] Input molar ratio of HMG 17/200 base pairs of DNA.

\[^{b}\] Bound molar ratio of HMG 17/200 base pairs of DNA.
**HMG 17-Chromatin Interaction**

**TABLE III**

Circular dichroism results of whole chromatin, stripped chromatin, and their HMG 17 reconstitutes

Stripped chromatin is H1/H5-depleted chromatin. All samples were in 0.25 mM EDTA, 1.0 mM Na phosphate, pH 7.0, and spectra were taken at 23 °C.

| Sample                  | HMG Input†  | Bound‡ | NaCl mol | [θ]207.5° | [θ]272.5° |
|-------------------------|-------------|--------|----------|------------|-----------|
|                         | 0 mol m.M   | 0 mol  | -        | 4100       | 3700      |
| Whole chromatin         | 1 0.40      | 0 50   | 3700     | 3300       |           |
|                         | 2 0.88      | 0 50   | 3400     | 2900       |           |
|                         | 0 0         | 50     | 2900     | 2400       |           |
|                         | 1 0.40      | 50     | 2900     | 2100       |           |
|                         | 2 0.88      | 50     | 2500     | 1700       |           |
| Stripped chromatin      | 0 0         | 0 50   | 5000     | 4400       |           |
|                         | 1 0.46      | 0 50   | 4600     | 3900       |           |
|                         | 2 0.84      | 0 50   | 4100     | 3400       |           |
|                         | 0 0         | 50     | 4300     | 4200       |           |
|                         | 1 0.46      | 50     | 3800     | 3300       |           |
|                         | 2 0.84      | 50     | 3800     | 3200       |           |

† Input mole ratio of HMG 17/200 base pairs of DNA.
‡ Bound mole ratio of HMG 17/200 base pairs of DNA derived from Table I.

The CD spectrum of chromatin above 250 nm has a lower ellipticity than native DNA. Cowman and Fasman (32) interpreted the chromatin CD spectrum in the nucleotide region as being composed of two independent components. One component is a conventional B-type DNA spectrum and the other, a Ψ-type spectrum with a negative [θ]272 contribu-
tion due to DNA wrapped around the core histones (33). The protein-free linker DNA gives a simple B-type spectrum with a positive [θ]260 deg cm²·dmol⁻¹· nucleotide. Another explanation for the decrease in [θ] values of DNA in chromatin is attributed to a change in the winding angle of DNA when it is wrapped around core histones in chromatin (34), i.e. a secondary structural change. Since the binding of HMG 17 to whole chromatin is accompanied by a further reduction in [θ] values in the nucleotide regions of both native whole chromatin and core chromatin, it is assumed that an additional Ψ band contribution or change in winding angle of additional DNA is produced in the HMG-bound complexes of chromatin. This would be in agreement with data from thermal denaturation which indicated a loss of DNA from the linker region upon binding of HMG 17. This implies that some unbound DNA becomes associated with the core upon HMG 17 binding, but other conformational changes resulting in a reduction of ellipticity cannot be excluded. The magnitude of the reduction in the [θ]260,5 value was found to be essentially the same for native whole chromatin (from 4100 to 3400 deg cm²·dmol⁻¹) and for stripped chromatin (from 5000 to 4100 deg cm²·dmol⁻¹) on binding =1 mol of HMG 17/nucleosome. This means that the Ψ-type band contribution or change in the winding angle produced by HMG 17 is essentially the same in whole and core chromatin, although there are additional conformational changes caused by H1 in whole chromatin.

**Circular Dichroism Studies: High Ionic Strength**

Recent reports have shown that nucleosomes and chromatin can assume different conformations depending upon the salt concentration (20, 26, 35, 36). Therefore, the binding of HMG 17 to whole chromatin and H1-H5-depleted chromatin has been studied in a medium containing 50 mM NaCl, where differences in condensation have been shown to exist relative to low ionic strength (31). In addition, it has been reported that there are different binding sites for HMG 17 in chromatin effective at various ionic strengths (11, 15).

**CD Studies of HMG 17-reconstituted Whole Chromatin**

The CD spectra of the HMG 17 reassembled whole chromatin complex and control whole chromatin in a medium containing 50 mM NaCl, 0.25 mM EDTA, 0.1 mM Na phosphate, pH 7.0, were studied (Fig. 5). Above 50 mM NaCl, chromatin slowly started precipitating, so that CD spectrum could not be taken. Control whole chromatin had ellipticity peaks at [θ]207,2 and [θ]272, with values of 2900 and 2300 ± 300 deg cm²·dmol⁻¹, respectively (Table III). When approximately 1 molecule of HMG 17 was bound for every 2 nucleosomes (0.40 mol/nucleosome), the CD spectrum of the whole chromatin was unaffected, indicating that no condensation had occurred. When 1 molecule of HMG 17 was bound for every nucleosome (0.88 mol/nucleosome), the [θ]207,2 decreased to 2500 deg cm²·dmol⁻¹, thus indicating some compaction of structure. Thus, it would appear that for condensation to occur at higher ionic strength it is necessary to have HMG 17 associated with each nucleosome. This might imply an interaction between neighboring nucleosomes at higher ionic strengths. From the data presented in Table III it is possible to determine whether the binding sites are all equivalent. Kuehl et al. (11) have shown that chromatin contains several classes of HMG 17 binding sites with different association constants. The data presented above may be viewed in the light of possibly two
low ionic strength (Fig. 6). When 0.46 mol of HMG 17 was bound/200 base pairs of DNA of striped chromatin, a decrease in $\theta_{280}$ of about 500 deg cm$^2$ dmol$^{-1}$ was observed (Table III). When an additional amount of HMG-17 (0.84 mol of HMG 17) was bound/nucleosome no further decrease in ellipticity was observed. Thus, when $\approx$1 mol of HMG 17 was bound/2 nucleosomes in stripped chromatin, the HMG 17 caused compaction of the DNA; however, no further condensation occurred when the ratio of HMG 17 was increased to $\approx$1 mol/nucleosome. Thus, the mechanism of binding HMG 17 at 50 mM NaCl differs in whole chromatin and stripped chromatin. Therefore, H1 changes the mode and effect of binding of HMG 17 at higher ionic strength.

The $\theta_{280}$ for whole chromatin was lower than that found for a reconstitute of 0.84 mol of HMG 17/nucleosome with stripped chromatin in 50 mM NaCl. Thus, if HMG proteins displace H1 in actively transcribing chromatin, a decondensation could occur.

Circular Dichroism Spectral Studies in the Protein Region—To investigate whether HMG 17 produces conformational changes in the core histones upon binding, CD spectral measurements were carried out in the protein region from 250 to 200 nm. The region below 250 nm is mainly due to the optical activity of the histone peptide chromophore. The magnitude of ellipticities at 208 and 220 nm is a measurement of the secondary structure of the core protein (17). Identical CD spectra were observed below 250 nm for both HMG 17-reconstituted core chromatin and the control core chromatin under identical conditions. Thus, binding of HMG 17 does not alter the conformation of the core histones.

CONCLUSIONS AND SUMMARY

The binding of HMG 14/17 to mononucleosomes has been shown to produce two major additional slow moving bands in nondenaturing polyacrylamide gels (14–16) indicating the binding of 1 and 2 molecules of HMG 14/17 per nucleosome. The reaction of HMG 14/17 with nucleosomes at a low ionic strength was found to be reversible and noncooperative. The mechanism of HMG 17 binding was dependent upon ionic strength of the medium. At a higher ionic strength, only one additional band of HMG 17 bound nucleosomes was found in the polyacrylamide gels (2 molecules of HMG 14/17 per nucleosome). Thus, the interaction of HMG 17 with nucleosomes at higher ionic strength was cooperative in nature (15). Differences in binding at low and high ionic strength were also obtained in our CD studies.

Goodwin et al. (8) postulated that in active chromatin, H1 is replaced by the binding of the NH$_2$-terminal basic regions of HMG 14/17 to 10–20 base pairs of DNA immediately contiguous to the core region and the COOH-terminal region binds to the core particle itself, weakening the histone DNA interaction in that region. The DNase I digestion studies on poly(dA-dT)-reconstituted core histone complexes containing HMG 14 showed altered digestion rates at selected sites which is in agreement with the above suggestion (15). However, Sandeen et al. (15) demonstrated that HMG 14/17-stabilized mononucleosomes as detected by thermal denaturation studies and concluded that HMG 14/17 bound to 20–25 base pairs at the end of the core particle. The results obtained herein from the thermal denaturation studies indicate that the binding of HMG 17 occurs to both the linker DNA and to the DNA contiguous to the core region of chromatin and that it stabilizes the structure. HMG 14/17 can be cross-linked to both H1 and the core histones, even with a "zero length" condensing agent (38). This again suggests that the position of HMG 14/17 is contiguous with the nucleosome core and H1 in chromatin. Thus, binding of HMG 17 need not interfere

![CD Spectra](image)

**FIG. 5.** Circular dichroism spectra of native whole chromatin and its complexes with HMG 17. Solution contained in 50 mM NaCl, 1.0 mM Na phosphate, 0.25 mM EDTA, pH 7.0. Whole chromatin (—). Bound molar ratios of HMG 17/200 base pairs of DNA: 0.4 (— —) and 0.88 (— —). Cell path length 1 cm; $A_{258}$ of chromatin $\approx$1.0; temperature 23 °C.

**FIG. 6.** Circular dichroism spectra of H1–H5-depleted chromatin and its complexes with HMG 17. Solution contained in 50 mM NaCl, 1 mM Na phosphate, 0.25 mM EDTA, pH 7.0. H1–H5-depleted chromatin (—). Bound molar ratio of HMG 17/200 base pairs of DNA: 0.46 (— —) and 0.84 (— —). Cell path length 1 cm; $A_{258}$ of stripped chromatin $\approx$1.0; temperature 23 °C.

CD Studies of HMG 17-reconstituted H1–H5-depleted Chromatin—The CD spectrum of H1–H5-depleted chromatin in 50 mM NaCl was very similar to that of whole chromatin at different binding sites.

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in any way with H1 binding in chromatin. The CD and thermal denaturation studies herein indicated that a condensation and stabilization of chromatin structure was produced by HMG 17 binding, which is in agreement with the reduced DNase I sensitivity of bulk nucleosome-HMG 14/17 complexes (15).

From the above discussion, it is evident that the question remains to be answered as to how HMG 14/17 confers special DNase I sensitivity to active gene sequences in chromatin. It should be pointed out that an overall stabilization of chromatin does not necessarily mean the inhibition of RNA polymerase binding to local segments of chromatin. HMG 17 may be involved in the modification of the internucleosomal interactions. Weinstein (38) has proposed a model wherein the condensation of chromatin is necessary to align recognition sites for RNA polymerases. Another explanation may be that the postsynthetic modifications of histones such as acetylation, phosphorylation, and methylation (39), coupled with the binding of HMG 14/17 produce the necessary conformational changes for transcription. In addition, HMG 14/17 can undergo postsynthetic modification, such as ribosylation by poly(ADP ribose) (40), acetylation (41), methylation (42), and phosphorylation (43). Thus, the modification of HMG 17 may also have a role in their association with the active gene.

From the results obtained herein, there was no evidence to suggest a weakening of internucleosomal interactions in high molecular weight chromatin, which would have been undetectable in the previous monomer studies. It has been shown that HMG 17 causes an overall compaction and stabilization of native chromatin and H1–H5 depleted chromatin, and it is evident that the binding of HMG 17 to chromatin, as studied herein, would not be responsible for the increased DNase I sensitivity found in active genes.

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Note Added in Proof—McGhee et al. (44) have demonstrated that HMG 14 and 17 do not induce an extended configuration in bulk or chromatin of the chicken β-globin gene.

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