Structural and Transcriptional Properties of Different Nucleosomal Particles Containing High Mobility Group Proteins 14 and 17 (HMG 14/17)*

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Binding of high mobility group (HMG) proteins 14 and 17 (HMG 14/17) to complete nucleosomal cores and to cores lacking one H2A-H2B dimer, the amino-terminal tails of histones, or both one H2A-H2B dimer and the amino-terminal ends of histones is accompanied by an overall stabilization of the particles as determined by thermal denaturation, circular dichroism and DNase I digestion. In spite of the structural stabilization brought about by HMG 14/17, the presence of these proteins causes little effect on the efficiency of the different nucleosomal particles as transcription templates for RNA polymerase II. The nucleosomal particles lacking one H2A-H2B dimer and containing two bound HMG 14/17 molecules are efficient in vitro transcription templates, which allow transcription of the whole length of the DNA present in the particle. These results are consistent with HMG 14/17 being present in active chromatin.

The non-histone proteins HMG 14 and 17 appear to be associated to chromatin active in transcription (1, 2). However, very little is known about the way in which they may affect this process. In vitro, HMG 14/17 binds strongly to nucleosomal cores, where there are two equivalent binding sites (3–6). These sites are located near the two ends of nucleosomal core DNA (4, 6). Binding of two HMG 14/17 molecules to the nucleosomal core causes stabilization of the particle against thermal denaturation (5, 7, 8), which is the opposite effect of what might be expected if these proteins facilitate transcription. Recently, an attempt has been made to evaluate the effects on transcription of different structural features of the nucleosomal particle (9, 10). Using an in vitro system containing isolated mononucleosomal particles as transcription templates, it has been shown that the loss of one H2A-H2B dimer from the nucleosomal particle is accompanied by a substantial increase in transcription efficiency. In contrast, elimination of the amino-terminal tails of histones, which causes a protein loss and a structural relaxation similar to those obtained by the absence of one H2A-H2B dimer, does not alleviate the histone octamer block to transcription. In the present work, we have investigated the structural effects produced by the binding of HMG 14/17 to nucleosomal cores lacking one H2A-H2B dimer, the amino-terminal ends of histones, or both, and the efficiencies of the HMG 14/17-containing particles as in vitro transcription templates.

Experimental Procedures and Results

The relative affinities of HMG 14/17 for intact and modified nucleosomal cores were evaluated by determining the distribution of limiting amounts of HMG 14/17 between H1/H5-deprived oligonucleosomes and the mononucleosomal particles being studied (Table I). The structurally altered nucleosomal cores employed lacked one H2A-H2B dimer (H2A-H2B-deficient cores), the amino-terminal tails of histones (amino-terminal-deficient cores), or both one H2A-H2B dimer and the amino-terminal tails of histones (H2A-H2B-amino-terminal-deficient cores). The absence of a change in affinity for HMG 14/17 of the nucleoprotein elements present in oligonucleosomes, with respect to nucleosomal cores (Table I), is in contrast with the reported large increase in affinity which accompanies a small increase in the length of the DNA present in the nucleosomal particle (11). The observed affinities of HMG 14/17 for complete and H2A-H2B-deficient cores are similar (Table I). It has been reported that HMG 14/17 binds preferentially to nucleosomal particles containing actively transcribed DNA sequences (12). These nucleosomal particles are supposed to have an altered structure required for transcription. Haer and Rhoads (13) proposed that the nucleosomal particles present in transcriptionally active chromatin might be deficient in histones H2A and H2B and that the two molecules of HMG 14/17 which bind specifically to nucleosomal particles might replace one H2A-H2B dimer. The absence of a difference in the affinity of complete and H2A-H2B-deficient cores for HMG 14/17 (Table I), and the different electrophoretic mobilities of the complexes between the two types of nucleosomal particles and HMG 14/17 (Fig. 3, Miniprint), suggest that two molecules of HMG 14/17 do not replace one H2A-H2B dimer in the nucleosomal particle and that the loss of one H2A-H2B

* This work was supported in part by the Dirección General de Investigación Científica y Técnica (PB6384), Spain and by an institutional grant from the Fundación Areces. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a fellowship from the Fondo de Investigaciones Sanitarias de la Seguridad Social, Spain.
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1 The abbreviation used is: HMG, high mobility group.

Prepared in U.S.A.
preferential binding to HMG 14/17. The loss of the amino-dimer from the nucleosomal cores is not sufficient to induce terminal tails of histones produces a substantial increase in suggests that the amino-terminal domains of histones mod-

TABLE I

Relative affinities toward HMG 14/17 of different structurally altered nucleosomal cores (competition with oligonucleosomes for binding to HMG 14/17)

Each type of mononucleosomal particle (100 μg DNA/ml) was mixed with oligonucleosomes (trinucleosomes and tetraneucleosomes, 117 μg DNA/ml), which contained the same number of nucleoprotein elements as the mononucleosomal particles, and with 3H-labeled HMG 14/17 (one HMG 14/17 molecule/nucleoprotein element present in the final mixture). The preparation was centrifuged in a linear sucrose gradient, as described in the legend to Fig. 4, Miniprint, and the distribution of [3H]HMG 14/17 between the oligonucleosomal and the mononucleosomal fractions was determined.

<table>
<thead>
<tr>
<th>Nucleosomal particles</th>
<th>HMG 14/17 bound</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Complete cores</td>
<td>46</td>
</tr>
<tr>
<td>H2A-H2B-deficient cores</td>
<td>48</td>
</tr>
<tr>
<td>Amino-terminal-deficient cores</td>
<td>68</td>
</tr>
<tr>
<td>H2A-H2B-amino-terminal-deficient cores</td>
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The loss of the amino-dimer from the nucleosomal cores is not sufficient to induce preferential binding to HMG 14/17. The loss of the amino-terminal tails of histones produces a substantial increase in the affinity of the nucleosomal core for HMG 14/17, which suggests that the amino-terminal domains of histones modulate the interaction of nucleosomal cores with HMG 14/17.

In all the nucleosomal particles studied in this work, the binding of HMG 14/17 causes an overall structural stabilization. The thermal denaturation studies show significant increases in the transition midpoints (T_a and T_b, Table II) upon binding of two molecules of HMG 14/17 to the nucleosomal particles, as well as a transfer of DNA base pairs from the low to the high temperature transition. The presence in the nucleosomal particles of HMG 14/17 produces, in all cases, a small decrease in ellipticity at 275 nm, which corresponds to an increase in DNA condensation (Table II). In agreement with these results, HMG 14/17 protects the DNA of the modified particles against DNase I digestion (Fig. 7, Miniprint). However, a slight increase in the susceptibility to digestion of intact cores is observed upon binding of HMG 14/17, this effect being much smaller than the one reported previously (14).

Fig. 1 shows the levels of RNA synthesized by RNA polymerase II using nucleosomal templates containing HMG 14/17 as transcription templates. In spite of the described stabilization by HMG 14/17 of the different nucleosomal structures, these non-histone proteins have little or no effect on the transcription levels of the particles (see Ref. 10). In particular, the H2A-H2B-deficient cores maintain their increased transcription efficiency in the presence of HMG 14/17. Moreover, Fig. 2 shows that the presence of HMG 14/17 does not prevent transcription of the whole DNA template of H2A-H2B-deficient cores, whereas with intact cores the block to transcription remains in the presence of HMG 14/17. Using RNA polymerase from Escherichia coli, the results (Fig. 9, Miniprint) show that the incorporation of [3H]UTP into RNA was determined after the indicated incubation times. The template concentration was 4 μg DNA/ml, and the molar ratio of RNA polymerase II to particle was equal to 2 ×, free DNA (14 base pairs); ◊, nucleosomal cores; ▲, H2A-H2B-deficient cores; ◼, amino-terminal-deficient cores; and ■, H2A-H2B-amino-terminal-deficient cores. All assayed templates, except free DNA, contained HMG 14/17. Addition of the corresponding amount of HMG 14/17 to free DNA has no effect on RNA synthesis (not shown). After 50-min incubation, seven UTP molecules were incorporated into RNA per molecule of free DNA (×).

TABLE II

Effect of HMG 14/17 on the thermal denaturation and circular dichroism parameters of different nucleosomal particles

T_a, transition midpoint, is the temperature of maximum dH/dt. H, hyperchromicity. The a and b (in parentheses) designate the two thermal transitions. F, fraction of altered DNA structure contributing to the circular dichroism difference band; equal to the ratio of the sample difference band intensity, [$\theta_{275,\text{sample}}$] - [$\theta_{275,\text{DNA}}$], to the core particle difference band intensity, [$\theta_{275,\text{core}}$] - [$\theta_{275,\text{DNA}}$]. The value for [$\theta_{275,\text{DNA}}$, 9300 deg X cm²/dmol nucleotide, was taken from Cowman and Fasman (15).

<table>
<thead>
<tr>
<th>Nucleosomal particles</th>
<th>HMG 14/17 [particle]</th>
<th>T_a(a)</th>
<th>F_a</th>
<th>T_a(b)</th>
<th>F_b</th>
<th>F</th>
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<tr>
<td>Complete cores</td>
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<td>75</td>
<td>66</td>
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<tr>
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<td>50</td>
<td>45</td>
<td>0.61</td>
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</tr>
<tr>
<td>Amino-terminal-deficient cores</td>
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<td>62</td>
<td>70</td>
<td>51</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>H2A-H2B-amino-terminal-deficient cores</td>
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<td>56</td>
<td>50</td>
<td>56</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>62</td>
<td>70</td>
<td>53</td>
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</tr>
<tr>
<td></td>
<td>0</td>
<td>53</td>
<td>62</td>
<td>35</td>
<td>0.32</td>
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</table>

Fig. 1. RNA synthesis by RNA polymerase II using different HMG 14/17-containing nucleosomal templates. The incorporation of [3H]UTP into RNA was determined after the indicated incubation times. The template concentration was 4 μg DNA/ml, and the molar ratio of RNA polymerase II to particle was equal to 2 ×, free DNA (14 base pairs); ◊, nucleosomal cores; ▲, H2A-H2B-deficient cores; ◼, amino-terminal-deficient cores; and ■, H2A-H2B-amino-terminal-deficient cores. All assayed templates, except free DNA, contained HMG 14/17. Addition of the corresponding amount of HMG 14/17 to free DNA has no effect on RNA synthesis (not shown). After 50-min incubation, seven UTP molecules were incorporated into RNA per molecule of free DNA (×).

Fig. 2. Size distribution of the RNA molecules synthesized by RNA polymerase II using nucleosomal templates containing HMG 14/17. Densitometric tracings obtained after electrophoresis of the RNA molecules synthesized with the indicated templates. Template concentration was 4 μg DNA/ml, and the molar ratio of polymerase to template was equal to 2. Incubation took place for 50 min.
Nucleosomal Particles Containing HMG 14/17

print) were entirely similar to those corresponding to RNA polymerase II (Fig. 1), which indicates that binding of HMG 14/17 does not inhibit the facilitated transcription obtained with the H2A·H2B-deficient cores. The non-histone proteins HMG 14/17 have been associated to chromatin active in transcription (1, 2). Our results indicate that binding of HMG 14/17 to H2A·H2B-deficient cores does not produce any detectable effect on the in vitro transcription properties of the particle, in spite of the significant structural stabilization. Therefore, since HMG 14 and 17 do not cause any negative effect on the in vitro transcription of H2A·H2B-deficient cores, these proteins might be present in nucleosomal particles during in vivo transcription, where they probably fulfill unknown functions not detected with the employed assays.

Acknowledgments—We thank Santiago Arenas for skillful technical assistance and Francisco Montero (Universidad Complutense de Madrid) for making available to us the Jobin-Yvon Mark III dicrograph. Chicken blood was kindly donated by AVESUR, Madrid.

REFERENCES

SUPPLEMENTARY MATERIAL TO:
STRUCTURAL AND TRANSCRIPTIONAL PROPERTIES OF DIFFERENT NUCLEOSOMAL PARTICLES
PUBLISHED IN THE JOURNAL...

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EXPERIMENTAL PROCEDURES

Preparation of Nucleosomal Particles. Nucleosomal cores and mononucleosomes lacking histones H1 and H5 were prepared from chicken erythrocyte nuclei, isolated after lysis of the cells in the presence of sodium PMSF (16). Nuclei were digested with micrococcal nuclease and extracted with 0.25 M NaCl (pH 6.5) (17). Nucleosome particles containing H1 and H5 were precipitated from the extract with 100 mM NaCl, and the nucleosomal cores were isolated from the supernatant (17). The precipitated H1/H5-containing particles were resuspended in 10 mM Tris-HCl (pH 8.0) containing 5 mM EDTA, and dialyzed overnight at 4°C against the same buffer solution (18). From the dialyzed preparation, an oligonucleosome fraction was isolated by centrifugation in a 5-20% sucrose gradient containing the components of the resuspension buffer solution. To increase the resolution of the oligonucleosome preparation was dialyzed against 10 mM Tris-HCl (pH 8.5), 0.1 mM DTT, and 5 mM EDTA (19). The oligonucleosome particles were separated from the released histones by sucrose gradient centrifugation in the presence of 0.7 M NaCl.

H1·H2B-deficient cores were obtained from the complete cores by treatment with dimethylaminopropylamine (DAPA). Nucleosomes lacking histones H1 and H2A·H2B·H5·H14·H17-deficient cores were prepared by gel permeation of complete and H1·H2B-deficient cores, respectively, as previously described (17).

The HMG 14/17-containing particles were isolated by incubation of the different nucleosomal particles with HMG 14/17 in 10 mM Tris-borate (pH 8.0), 1 mM EDTA, for 30 min at 4°C. Unless otherwise indicated, the mixtures contained 0.25 μg of HMG 14/17 per μg of DNA. The HMG 14/17-containing particles were isolated by centrifugation in a linear 5-20% sucrose gradient containing 80 mM Tris-borate (pH 8.0) and 3 mM EDTA. Pretreating HMG 14 and 17 were purified from rat thymus essentially as described by Murr. 3H-Labeled HMG 14 and 17 were purified by reductive alkylation using [3H]-

Thermal Denaturation. The denaturation profiles of the nucleosomal particles, in 0.25 mM EDTA (pH 8.0), were obtained with a Hitachi 240 spectrophotometer equipped with a 2527 thermostate and a programmed printer. Absorbance at 260 nm was registered at 10 s intervals between 25 and 95°C, using a heating rate of 1°C/min. The derivative (ΔΔAbs) curves, obtained as previously described (21), were resolved into component thermal transitions by Gaussian curve fitting with a NONMEM nonlinear regression program.

Circular Dichroism. Spectra of nucleosomal particles (25 μg DNA/ml), in 0.25 mM EDTA (pH 8.0), were obtained with a Mark IIIR dichrograph (Jasco-Prior), using a sensitivity of 10-6 A/cm, and cells with an optical path of 1.0 cm.

Digestion with DNase I. The nucleosomal particles, in 10 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, and 8 mM CaCl₂, were digested at 0.4°C with pancreatic deoxyribonuclease I (Dnase I, type 1, Sigma). The reaction was ended by addition of EDTA to a concentration of 10 mM. The DNA fragments were separated from the protein (22), and subjected to electrophoresis in gels containing 10% polyacrylamide and 7 M urea (23).

Transcription Assay. Transcription was estimated by measuring the radioactivity from [3H]-UTP incorporated into acid-precipitable RNA as previously described (18, 20). The size of the transcription products was determined by electrophoresis of the purified RNA in gels containing 10% polyacrylamide and 7 M urea (23).
Nucleosomal Particles Containing HMG 14/17

RESULTS

Binding of HMG 14/17 to Nucleosomal Particles. Two molecules of HMG 14/17 were added for each nucleosomal particle. Under the employed conditions, all the HMG 14/17 molecules are bound to the particles, as determined by sucrose gradient centrifugation using \(^{14}C\)-labeled HMG 14/17. The binding of HMG 14/17 to the nucleosomal particles does not cause any detectable change in the sedimentation coefficient of the particles (not shown). Electrophoresis under non-denaturing conditions of the complexes formed between HMG 14/17 and complete and H3A-H2B-deficient cores shows a decrease in mobility induced by the binding of HMG 14/17 (Fig. 3).

Fig. 3. Electrophoresis of complete and H3A-H2B-deficient nucleosomal cores in the presence and absence of HMG 14/17. Complete nucleosomal cores with (b) and without (a) HMG 14/17; H3A-H2B-deficient cores with (d) and without (c) HMG 14/17; and free DNA 146 base pairs (e).

To evaluate the relative affinity of HMG 14/17 for the different nucleosomal particles, each type of nucleosomal particle was incubated with oligonucleosomes (tri and tetranucleosomes deprived of histones H1 and H5) containing the same amount of nucleoprotein elements as the nucleosomal particles, and \(^{14}C\)-labeled HMG 14/17 (one molecule of HMG 14/17 for each nucleoprotein element in the mixture). The distribution of radioactivity between mono- and oligonucleosomes was determined after separation of particles in a sucrose gradient. Fig. 4 shows the distribution of radioactivity between oligonucleosomes and H3A-H2B-deficient cores, the results corresponding to the different, mono-nucleosomal particles being shown and discussed in the main section of this work (Table 1).

Fig. 4. Comparison for binding of HMG 14/17 between oligonucleosomes and H3A-H2B-deficient cores. Sedimentation patterns of \(^{14}C\)-labeled HMG 14/17 and H3A-H2B-deficient cores (127 µg DNA/mi) (A), H3A-H2B-deprived oligonucleosomes (127 µg DNA/mi) (B), and both H3A-H2B-deficient cores (100 µg DNA/mi) and oligonucleosomes (127 µg DNA/mi) (C). The concentration of \(^{14}C\)-labeled HMG 14/17 was 27 µg/ml, approximately 1 molecule of HMG 14/17 for each nucleoprotein element. The nucleosomal particles were incubated with \(^{14}C\)-HMG 14/17 at 4°C for 15 min, in 10 mM Tris-Cl pH 8.3, 15 mM KCl, and 1 mM EDTA. The mixtures were centrifuged for 4 h at 4°C, in a linear 5-20% sucrose gradient containing 10 mM Tris-Cl pH 8.3, 15 mM KCl, and 1 mM EDTA. Centrifugation took place at 24,000 rpm in an SW50.1 rotor. After centrifugation, the gradients were fractionated (150 µl fractions), and the radioactivity of the fractions was measured. The arrow in B shows the position of free DNA (146 base pairs). In C, the arrow shows the position of the complexes A and B.

Structural Properties of the Different Nucleosomal Particles. The thermal denaturation profiles of the different nucleosomal particles are shown in Fig. 5. With instant nucleosomal cores, the first thermal transition (Tm = 62°C) corresponds to the melting of the DNA ends, and the second (Tm = 57°C) to that of central DNA (D5). The loss of one H3A-H2B dimer is accompanied by lowering of the two transition midpoints, and an increase in the fraction of DNA melting in the first transition, in agreement with previous reports (15,28,29). Elimination of the mono-nucleosomal ends of histones by trypsin digestion, produces changes in the thermal denaturation parameters similar to those corresponding to the loss of one H3A-H2B dimer (Table 1). When both structural alterations, loss of one H3A-H2B dimer and that of the mononucleosomal tails of histones, are simultaneously present, nucleosomal DNA is largely destabilized, with approximately 95 base pairs melting in the first transition. Binding of two molecules of HMG 14/17 to each nucleosomal particle is accompanied, in all cases, by stabilization of DNA, with increase in the midpoints of the two transitions and in the fraction of DNA melting in the higher transition.

Fig. 5. Thermal denaturation profiles of complete and deficient nucleosomal cores in the presence (-----) and absence (-----) of HMG 14/17.

According to Comas and Fassan (15,26), the circular dichroism spectrum of nucleosomal cores from 250 to 320 nm can be resolved into two components, the positive band corresponding to the secondary structure of free DNA (B form), and a negative band centered at 275 nm produced by the renaturation of DNA into an asymmetric tertiary structure. Fig. 6 shows the spectra of the nucleosomal particles used in this work. In the absence of HMG 14/17, all the structural modifications introduced in the nucleosomal core are accompanied by a decrease in the negative component, corresponding to relaxation of the asymmetric tertiary structure of DNA. The largest change is observed in the H3A-H2B-ammonium-deficient particles, in agreement with the thermal denaturation studies. In all cases, binding of HMG 14/17 causes a decrease in ellipticity at 375 nm corresponding to a gain in DNA condensation. The largest effect is found in particles lacking one H3A-H2B dimer, whether renatured or not.

Fig. 6. Circular dichroism spectra of complete and deficient nucleosomal cores in the presence (-----) and absence (-----) of HMG 14/17.

The digestion pattern of nucleosomal particles with sequence 1 can detect small structural alterations (27). In Fig. 7, the patterns of digestion for the different nucleosomal particles are shown. Addition of HMG 14/17 to intact nucleosomal cores causes a slight decrease in the amount of the longer DNA fragments, which indicates that the presence of HMG 14/17 facilitates digestion. This effect is smaller than the one previously reported (15). The deficient particles investigated are most susceptible to digestion than the complete nucleosomal core, and the corresponding patterns show a decrease in the amounts of the longer fragments. With all their deficient particles, the presence of HMG 14/17 protects DNA from digestion; the proportion of the longer DNA fragments being increased with respect to the shorter ones. Therefore, except with complete nucleosomal cores, HMG 14/17 causes a significant protection of nucleosomal particles against digestion by Phase I.
When the 5' ends of DNA were labeled with $^{32}$P, the distribution of labeled DNA fragments shown in Fig. 5 were obtained after DNAse I digestion of complete and H2A/H2B-deficient cores, in the presence and absence of HMG 14/17. Both types of particles, the proportion of the larger fragments is much higher in the presence of HMG 14/17, without HMG 14/17, most of the longer DNA fragments were deprived of the labeled 5' end, not being detected in this experiment, in spite of being clearly seen in the experiment of Fig. 7. Apparently, HMG 14/17 binds near the labeled DNA ends, protecting them from digestion, in agreement with previous work using complete nucleosomal cores (1,6).

In vitro transcription with E. coli RNA polymerase. Fig. 9 shows the RNA synthesis obtained with the different HMG 14/17-containing particles and RNA polymerase from E. coli. The presence of HMG 14/17 has no significant effect on the levels of RNA synthesized (see Ref. 10), in agreement with the results obtained with RNA polymerase II from calf thymus (Fig. 11).
Structural and transcriptional properties of different nucleosomal particles containing high mobility group proteins 14 and 17 (HMG 14/17).

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