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The high mobility group proteins, HMG 14 and 17, do not prevent the formation of chromatin higher order structure

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ABSTRACT

The high mobility group proteins, HMG 14 and 17, have been associated with the chromatin of active genes (refs 1-8), although how they function is not known. We use sedimentation and electric dichroism to investigate the effect of HMG 14 and 17 on the condensation of chicken erythrocyte chromatin into higher order structure. We find no evidence that excess HMG 14 and 17 induce an extended configuration, either in bulk chromatin or in the chromatin of the chicken β-globin gene.

INTRODUCTION

The high mobility group proteins, HMG 14 and 17 (1) have been associated with differential gene expression in eukaryotes (2-8). In particular, Weintraub and co-workers (2-4) have demonstrated that HMG 14 and 17 confer the characteristic property of enhanced DNase I sensitivity on the chromatin of transcriptionally competent genes. Although HMG binding to bulk nucleosome core particles has been characterized in some detail (7, 9), it is not known how HMG 14 and 17 interact with polynucleosomes, especially those containing active genes. One possible mechanism by which HMG 14 and 17 could confer nuclease sensitivity on active genes and perhaps enhance their transcription, is by maintaining the active chromatin in an extended configuration i.e. by preventing the transition from 10 nm filament to 30 nm solenoid. We present evidence in this paper to rule out this hypothesis.

The filament ⇄ solenoid transition in bulk chicken erythrocyte chromatin can be monitored by the increasing sedimentation rate induced by increasing concentration of monovalent ions (see eg. ref 10), or by changes in electric dichroism properties induced by increasing divalent ions (11). We show that by either of these two assays, exogenous HMG 14 and 17 (at a level of 2 molecules of HMG per nucleosome or even higher) have little effect on the condensation of bulk chromatin. Using dot

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blot hybridization, we show that excess HMG 14 and 17 also do not prevent condensation of the adult β-globin genes in chicken erythrocytes.

MATERIALS AND METHODS

Chromatin fractions obtained from adult chicken erythrocytes were prepared as described previously (11). Erythrocyte nuclei from 14 day chicken embryos were prepared as described in (12), including centrifugation through 2 M sucrose, resuspended at an A_{260} ≈100 (or in one case, 200) in 100 mM NaCl, 50 mM Tris HCl, pH 8.0, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM Na butyrate, 0.1 mM PMSF and digested to 0.1-0.5% acid solubility with 0.01 units of micrococcal nuclease for 30 minutes at 37°C (nuclease units as defined in (13)). Digestion was stopped by addition of EDTA to 10 mM, and the chromatin released by overnight dialysis into 5 mM Tris HCl, 0.1 mM EDTA, 1 mM Na Butyrate, pH 8.0 and centrifugation at 8000 g for 10 minutes. The soluble chromatin (80-90% of nuclear DNA content) was used either unfractionated (see e.g. Fig 2) or after fractionation on an isokinetic sucrose gradient, containing 25 mM NaCl, 5 mM Tris HCl, 0.1 mM EDTA, 1 mM Na butyrate. Typical DNA gels are shown in Fig 1A. The protein composition of a typical chromatin preparation is shown in Fig 1B (18% polyacrylamide-SDS gel, stained with Coomassie Blue).

The high mobility group proteins HMG 14 and 17 were isolated from adult erythrocyte nuclei by extraction with 0.35 M NaCl and selective TCA precipitation, as described in more detail by Sandeen et al (7). Three independent preparations were used in this work and all gave equivalent results. A typical protein gel is shown in Fig 1C. (The notation HMG 14/17 is used to describe the approximately equimolar mixture of HMGs 14 and 17 resulting from the purification protocol; a ratio of 2 HMG 14/17 per nucleosome equivalent added to a chromatin preparation is thus equivalent to one molecule of HMG 14 and one molecule of HMG 17 added for every 210 base pairs of DNA).

The globin gene content of sucrose gradient fractions (Fig 4) was determined by a dot blot hybridization assay. Twenty μl of each gradient fraction (containing less than 3 μg of DNA) were mixed with 20 μl of 0.2 N NaOH, 2 mM EDTA, and allowed to sit at room temperature for 20 to 30 minutes. Seventy μl of 3.14 M $NaClO_4$, 1.57 M acetate buffer were added (i.e. to yield a final concentration of 2 M $NaClO_4$, 1 M acetate) and samples chilled in ice. (1 M acetate buffer was as described in (14) and contained 24.54 g sodium acetate·$3H_2O$ and

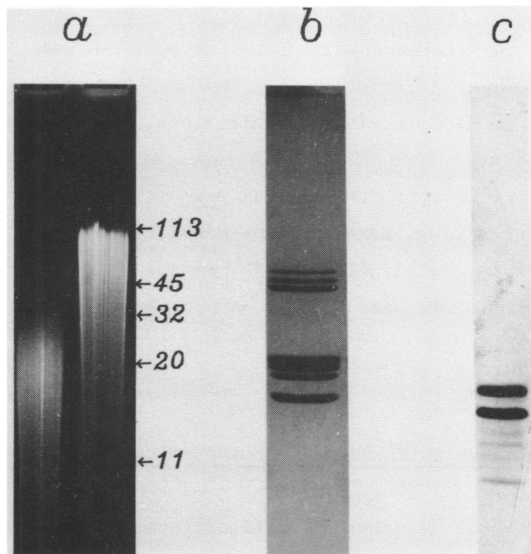

Fig. 1A. DNA content of two typical chromatin preparations. Agarose gel (1%) stained with ethidium bromide. DNA size markers (λ DNA restricted with Hind III) are expressed as multiples of 210 base pairs. The sample on the left was used in Fig 4 to measure globin gene sedimentation.

B. Protein content of a typical chromatin preparation.

C. Typical preparation of HMG 14/17.

47.6 ml glacial acetic acid per liter). DBM paper (Schleicher and Schuell) was prepared as described previously (12,14) and clamped into a Schleicher and Schuell "Minifold" filtration manifold, on top of several layers of filter paper soaked in cold 1 M acetate buffer. The paper was dried very lightly under mild vacuum (~1 sec) and 100 µl of each sample applied per well. The paper was again dried briefly under vacuum, 200 µl of cold 2 M $NaClO_4$, 1 M acetate were added, the paper dried again, (5-10 sec on mild vacuum), placed on several layers of filter paper soaked in cold 1 M acetate and stored overnight at 0-4°C in a glass tray sealed with Saran Wrap. Prehybridization and hybridization were exactly as described in (12). The hybridization probe was nick-translated 6.2 kbp EcoRI fragment, containing the chicken adult β-globin gene and isolated from the plasmid pCAβG1 (i.e. probe A of McGhee et al, (12)). Film densities were measured with a Joyce-Loebl densitometer. Each assay was usually done at least in duplicate and at least at two film exposures. Fairly large variations in the above dot blot protocol led to identical

conclusions.

Sedimentation coefficients were measured on a Beckman Model E analytical ultracentrifuge, interfaced to a Hewlett-Packard 1000 computer. To prepare samples at various salt concentrations, a concentrated chromatin solution was diluted into 20 to 60 volumes of solvent at the appropriate salt concentration, to give a final A_{260} of 0.4 to 0.6. HMG 14/17 were added by direct mixing, except where noted in the text. Sedimentation runs were performed at 12,000-16,000 rpm and a temperature of 17 to 18.5°C. Electric dichroism measurements were made at 2-4°C, essentially as described in Ref 11.

RESULTS

HMG 14/17 do not prevent bulk chromatin condensation as monitored by sedimentation

Soluble chromatin was released by mild micrococcal nuclease digestion of erythrocyte nuclei obtained from 14 day chicken embryos and was not fractionated with respect to size, in order to minimize possible losses of undefined components. Figure 2A (circles) shows the median sedimentation coefficient of this chromatin preparation as it increases with increasing salt concentration. At least over the salt range from 5 to 80 mM NaCl, the increase in $s_{20,w}$ is due to intramolecular compaction of of the chromatin into higher order structure (see e.g. Refs. 10, 15) and owes little to charge effects. This was verified by crosslinking a chromatin sample with 0.1% glutaraldehyde (15) in the presence of 50 mM NaCl; the sedimentation coefficient measured at ~1mM NaCl was only 5% lower than at 50 mM NaCl (data not shown), compared to a 35% decrease observed for uncrosslinked samples.

Figure 2A (triangles) shows the median $s_{20,w}$ obtained with this chromatin preparation in the presence of 2.0 ± 0.1 HMG 14/17 added per nucleosome. The added HMG 14/17 certainly do not prevent the rise in sedimentation rate associated with chromatin condensation and, in fact, cause a slight increase in $s_{20,w}$ (4.8%, averaged over all salt concentrations). An increase of ~5% in $s_{20,w}$ would be expected as the net effect of the larger mass and slightly increased partial specific volume, if there were no change in frictional properties. The dashed line in Fig 2A is the sedimentation behavior expected if this same chromatin preparation were stripped of histones H1 and H5 (estimated from Fig. 8 of ref. 10).

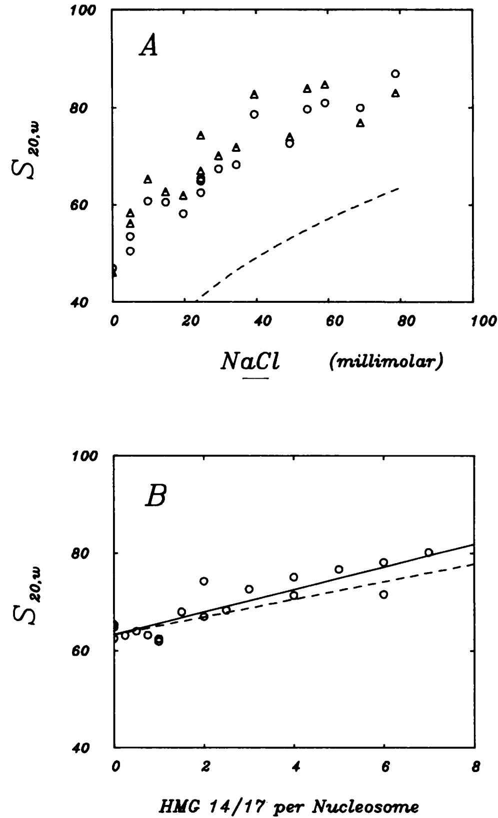

Fig. 2A. $s_{20,w}$, (the median sedimentation coefficient corrected to standard conditions) of chromatin as a function of added NaCl concentration. Unfractionated chromatin was obtained from 14 day erythrocytes and had a weight average DNA size of 26 nucleosomes, estimated from the sedimentation coefficient of the purified DNA. All solutions contained 5 mM Tris HCl, pH 8.0, 0.1 mM EDTA, 1 mM sodium butyrate. O = no added HMG 14/17; Δ = 2.0 ± 0.1 HMG 14/17 added per nucleosome;---- expected sedimentation behavior if histones H1 and H5 were removed.

B. $s_{20,w}$ plotted as a function of HMG 14/17 added per nucleosome, for the same chromatin preparation used in A. Solvent was 25 mM NaCl, 5 mM Tris HCl, 0.1 mM EDTA, 1 mM Na butyrate, pH 8. Solid line = best linear fit to data; dashed line = expected sedimentation behavior if all added HMG 14/17 were bound but caused no changes in the frictional properties of the chromatin particles.

From the data of Fig 2A, 25 mM NaCl corresponds roughly to the midpoint of the transition in $s_{20,w}$; sedimentation at this salt concentration should be maximally sensitive to conformational perturbations. Figure 2B shows that at 25 mM NaCl, up to 7 or 8 HMG 14/17 can be added

per nucleosome equivalent with no sign of saturation of the increase in $s_{20,w}$. The solid line in Fig. 2B is the least squares best fit to the data; the dashed line is the predicted relation if all HMGs bound, thereby increasing the particle mass and partial specific volume but not changing the frictional properties. Obviously HMG 14/17 do not cause any significant conformational changes in the chromatin solenoids. Even at 8 HMG 14/17 added per nucleosome, essentially all bind to the chromatin under these conditions, as verified by protein gels of sucrose gradient fractions (data not shown).

Results similar to those shown in Figure 2 have been obtained with four fractionated erythrocyte chromatin preparations (with sizes ranging from ~20 to ~80 nucleosomes), isolated either from 14 day embryos or adult chickens. An equimolar mixture of HMG 1,2,14 and 17 also caused only a small increase in $s_{20,w}$ (data not shown). Addition of HMG 14/17 to chromatin in 0.35 M NaCl followed by dialysis or dilution to 25 mM NaCl resulted in an ~7% increase in $S_{20,w}$, relative to a sample treated similarly but with no added HMG. However, the $s_{20,w}$ of this latter control sample is ~25% lower than that of the same chromatin preparation which has not been exposed to 0.35 M NaCl.

HMG 14/17 do not prevent bulk chromatin condensation as monitored by electric dichroism

Electric dichroism can be used to monitor the chromatin filament $\rightleftarrows$ solenoid transition induced by Mg^{++} ions (11). Figure 3 collects data obtained with four chromatin preparations (both fractionated and unfractionated, both from 14 day embryo and adult erythrocytes). Independently of the presence of 2.0 ± 0.1 HMG 14/17 added per nucleosome equivalent, essentially the same value of ρ_∞ (reduced dichroism extrapolated to infinite electric field) is obtained, both for the 10 nm chromatin filament (~-0.6 with no added divalent ion), and for the 30 nm chromatin solenoid (~-0.15 to -0.2 at 1 Mg^{++}/DNA phosphate). Field-free relaxation times (τ) reflect the overall particle dimensions and, as shown in Fig 3B, are essentially unchanged by the added HMG proteins.

Several of the data points in Fig. 3 were obtained with an equimolar mixture of HMG 1,2,14 and 17; no significant effect was observed. HMG 14/17 do not significantly alter the urea concentration (~3-4 M) at which condensed chromatin unfolds (data not shown), further evidence that HMG 14/17 do not perturb the solenoid stability. The HMG preparations contributed negligibly to solution conductivity.

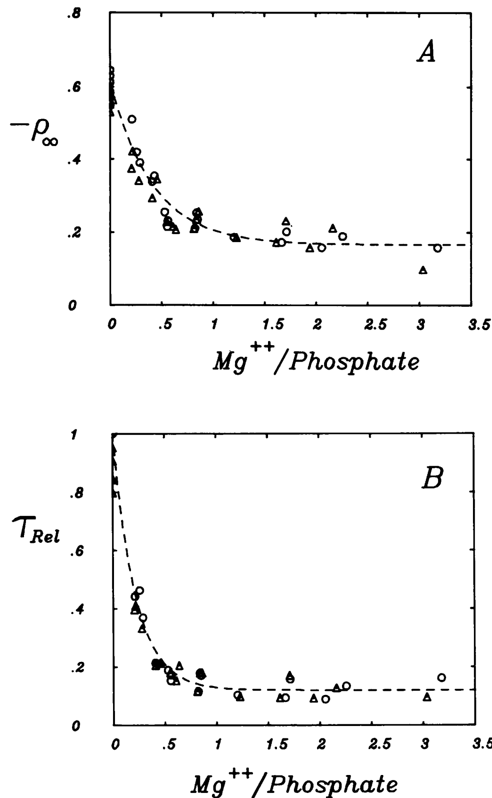

Fig. 3A. Reduced dichroism extrapolated to infinite electric field (ρ_{∞}) plotted as a function of Mg^{++} added per DNA phosphate. Data was combined from four separate chromatin preparations as described in text. O = no added HMG 14/17; Δ = 2.0 ± 0.1 HMG 14/17 added per nucleosome. Dashed line represents the arbitrary function: $y = ae^{bx}+c$, fit to the data obtained in the absence of added HMG 14/17.

B. τ_{Rel} (the average relaxation time normalized to the relaxation time of the uncondensed chromatin filament i.e. Mg^{++} = 0 and no added HMG 14/17), plotted as a function of Mg^{++} added per DNA phosphate. Chromatin preparations and symbols are the same as in Fig 3A.

HMG 14/17 do not change the condensation state of the chicken adult β-globin gene.

Chromatin obtained from 14 day erythrocytes was sedimented through an isokinetic sucrose gradient containing 0.35 M NaCl, in order to remove endogenous HMG 14/17. A fraction of size ~20 nucleosomes (the adult β-globin gene encompasses 8-10 nucleosomes) was dialyzed to 25 mM NaCl, either with or without 2 HMG 14/17 added per nucleosome. (This type of

reconstitution procedure has been shown by others (2-4,8) to restore DNase I sensitivity to active genes). The two samples were then sedimented through a second isokinetic sucrose gradient, containing 25 mM NaCl. The A_{260} profiles of these two gradients are shown in Fig 4A and, as expected, the sample containing HMG 14/17 sedimented marginally faster. Protein gels verified that HMG 14/17 were indeed bound on the

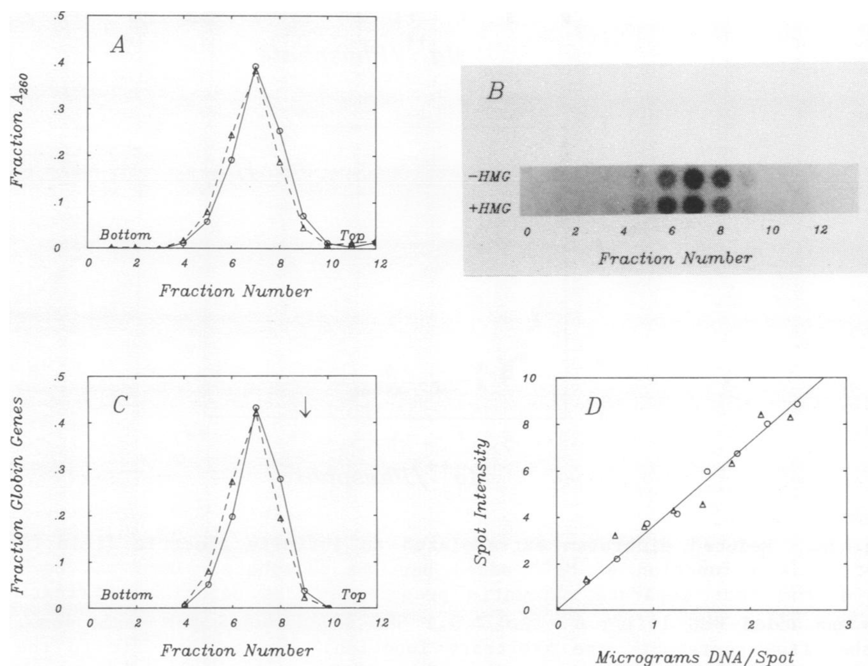

Fig. 4 Erythrocyte chromatin from 14 day embryos was stripped of endogenous HMG 14/17 and a size fraction containing ~20 nucleosomes sedimented on an isokinetic sucrose gradient containing 25 mM NaCl with or without added HMG 14/17. Direction of sedimentation is from right to left

A. Normalized A_{260} profile of gradients; O__O, no added HMG 14/17; Δ---Δ, +2 HMG 14/17 per nucleosome.

B. Typical dot blot hybridization assay measuring the adult β-globin gene content of each fraction of A.

C. Normalized globin gene content of gradients shown in Fig 4A. O__O = no added HMG 14/17; Δ---Δ = +2 HMG 14/17 per nucleosome. Arrow marks expected sedimentation position if this chromatin were stripped of histones H1 and H5.

D. Standard curve for dot blot assay, relating spot intensity (i.e. globin gene content in arbitrary units) to amount of chicken DNA applied per spot, either as chromatin (O) or as protein free DNA (Δ).

gradient (data not shown).

The amount of adult β-globin gene sequence contained in each fraction of the gradient in Fig 4A was determined by the dot-blot hybridization assay described in the Methods section and illustrated in Fig 4B. The linearity of the assay is demonstrated in Fig 4D. Fig 4C shows that for both chromatin samples, the globin gene cosediments with the absorbance, within experimental error. The arrow in Fig 4C marks the approximate sedimentation position expected if the chromatin were stripped of histones H1 and H5. Thus HMG 14/17 certainly do not prevent condensation of the adult β-globin gene.

HMG 14/17 also did not change the globin gene sedimentation in two other chromatin preparations (fractionated and unfractionated) which had not been exposed to 0.35 M NaCl (data not shown). These experiments also indicate that the adult β-globin gene is as condensed as the bulk chromatin, an important point which is more fully explored elsewhere (McGhee and Felsenfeld, manuscript in preparation).

DISCUSSION

Previous investigations have shown that isolated nucleosomes strongly bind two molecules of HMG 14/17 and that this binding is not prevented by the presence of the lysine-rich histones, H1 and H5 (7,9,16). Our present results show further that, wherever HMG 14/17 are bound in polynucleosomes, they do not significantly interfere with nucleosome packing in the 30 nm fiber, nor do they prevent important stabilizing interactions. The chromatin solenoid might thus be expected to be quite an "open" structure, allowing other molecules ready access to the DNA.

The current interest in HMG 14/17 arises, not from their interaction with bulk chromatin, but rather from their preferential interaction with active genes (2-8). However, we have found no evidence that HMG 14/17 induce an extended configuration in the chromatin of the chicken adult β-globin gene, in cells where this gene is known to be DNase I sensitive.

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