
Affinity of HMG17 for a mononucleosome is not influenced by the presence of ubiquitin-H2A semihistone but strongly depends on DNA fragment size

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ABSTRACT

We have used a two-dimensional (deoxyribonucleoprotein+DNA) electrophoretic binding assay to study the interaction of the purified high mobility group protein HMG17 with isolated HeLa mononucleosomes as a function of their DNA fragment size and the presence of ubiquitin-H2A semihistone. No significant differences between affinities of HMG17 for ubiquitinated and non-ubiquitinated core mononucleosomes were observed. In striking contrast, the apparent affinity of HMG17 for a mononucleosome increases more than 100-fold upon an increase of the length of the mononucleosomal DNA fragment by as few as 3 to 5 bp over the core DNA length (~146 bp). We suggest that the magnitude of this effect is sufficient to explain the preferential binding of HMG17 in vitro to mononucleosomes derived from actively transcribed genes.

INTRODUCTION

In vitro HMG-monomucleosome binding studies have established the existence of two specific binding sites for the high mobility group (HMG) proteins HMG14 or 17 on every core mononucleosomal particle [1-3]. When mononucleosomal preparations from chicken erythrocyte nuclei stripped of HMG proteins were partially titrated with HMG14/17, the nucleosome-HMG complexes were found to be enriched in β -globin DNA sequences [1]. Mononucleosomes derived from certain transcriptionally active genes, such as chicken globin genes in cells of erythroid lineage, were preferentially retained upon chromatography on HMG14/17 glass bead affinity columns [4-6]. These results confirmed and extended earlier findings which suggested a preferential association of HMG14/17 with transcribed genes in vivo [6-8]. The structural basis for preferential in vitro binding of HMGs to isolated "active" mononucleosomes remains unknown.

In separate studies we have recently found that a considerable proportion (~50%) of nucleosomes of transcribed Drosophila genes, such as copia and heat shock genes in non-shocked cells, contain ubiquitin-H2A semihistone (uH2A), a bifurcated covalent conjugate of histone H2A and a specific 76-residue protein, ubiquitin [10; see refs. 11, 12 for reviews on the chemistry and metabolism of uH2A]. In striking contrast, nucleosomes of tandemly repeated, nontranscribed 1.688 density satellite DNA are virtually devoid of uH2A semihistone [10], suggesting that nucleosomal uH2A is preferentially associated with transcribed or potentially transcribed genes.

One possibility raised by these findings [10] was that a substitution of uH2A for H2A in a nucleosome might increase its affinity for HMG14/17; this would explain a preferential association of HMGs with "active" nucleosomes by the presence of uH2A in the latter.

The results of in vitro HMG-monomucleosome binding experiments described below show that the presence of uH2A in a core mononucleosome does not significantly change its affinity for HMG17. At the same time, using a binding assay which allows high-resolution monitoring of DNA fragment size, we observed a striking dependence of the relative affinity of HMG17 for a mononucleosome on the size of its DNA fragment. We suggest that the magnitude of this dependence is sufficient to account for the preferential binding of HMG14/17 in vitro to mononucleosomes derived from active genes.

MATERIALS AND METHODS

Cell culture and preparation of chromatin

Monolayer cultures of HeLa S-3 cells were propagated in 15-cm plastic dishes (Lux) in Eagle's minimal essential medium with 10% calf serum (GIBCO). Cells were labeled with (Me-³H) thymidine (New England Nuclear; 20 Ci/mmol) at 10 μ Ci/ml for 15-20 hours at approximately 50% confluency in freshly added medium. Specific radioactivities of $1-2 \times 10^5$ ³H cpm/ μ g of DNA were obtained.

Labeled cell monolayers were rinsed with 0.14 M NaCl, 5

mM Tris-HCl at 4°C followed by addition of 0.5% Nonidet P40, 10 mM Na-EDTA, 5 mM Na-butyrate, 0.5 mM phenylmethylsulfonyl-fluoride (PMSF, freshly added from 0.5 M stock in absolute ethanol), 1 mM dithiobis (2-nitrobenzoic acid) (DTNB, Sigma, added from a freshly made 100 mM stock adjusted to pH 7.5 with 1 M Tris-HCl, pH 8.8), 10 mM Na-HEPES (pH 7.5). The lysate was scraped with a rubber policeman, homogenized by pipetting through a 10-ml Falcon pipette and then centrifuged at $\sim 1000\times g$ for 5 min. The pellet was resuspended and pelleted once more in the lysis buffer. The pellet obtained was resuspended and washed with 0.14 M NaCl, 10 mM Na-EDTA, 0.1 mM PMSF, 0.25 mM DTNB, 10 mM Na-HEPES (pH 7.5) followed by two 30-minute washes with 0.35 M NaCl, 10 mM Na-EDTA, 0.1 mM PMSF, 0.25 mM DTNB, 10 mM Na-HEPES (pH 7.5). Chromatin pellets were then washed twice with a digestion buffer (0.2 mM CaCl_2 , 0.1 mM PMSF, 0.1 mM DTNB, 1 mM Na-HEPES (pH 7.5)), and thereafter gently resuspended in the same buffer to approximately 0.5 mg of DNA per ml. Digestion with staphylococcal nuclease (Worthington) was at 2 $\mu\text{g}/\text{ml}$ for 20 minutes at 37°C. The reaction was stopped by addition of 0.02 vol. of 50 mM Na-EDTA, 25 mM Na-EGTA (pH 7.5). After incubation at 4°C for ~ 15 minutes the samples were centrifuged at 12,000 $\times g$ for 5 minutes. Supernatants were used either immediately or frozen at -70°C in the presence of 10% glycerol; freezing and thawing did not influence the electrophoretic patterns obtained.

DTNB, a thiol-specific reagent, was present throughout chromatin isolation and nuclease digestion because it inhibits uH2A isopeptidase, a PMSF-insensitive enzyme that cleaves uH2A into free ubiquitin and H2A [13; D. Finley and J. Barsoum, unpublished data]. DTNB is an inhibitor of a number of other chromatin-related enzymes as well, such as histone phosphatases and certain proteases [14]; it does not inhibit staphylococcal nuclease. Although the relative content of uH2A in isolated HeLa nucleosomes is not changed significantly upon omission of DTNB from chromatin isolation buffers, retention of uH2A in nucleosomes from some other cell lines critically depends on the presence of DTNB or other thiol-specific reagents during chromatin isolation (D. Finley and J.

Barsoum, unpublished data).

Purification of mononucleosomes

Nuclease-solubilized chromatin samples (0.5 ml) were layered onto 5-30% (w/v) linear sucrose gradients (11 ml) containing 1 mM Na-EDTA, 10 mM Na-HEPES (pH 7.5). The samples were centrifuged in an SW41 rotor (Beckman) at 39,000 rpm for 16 hours at 4°C. Fractions were collected from the bottom of a nitrocellulose tube and aliquots were counted with Aquasol (New England Nuclear).

Purification of HMGl7 from calf thymus

The procedure used was a slight modification of previously published methods [15]. Fresh calf thymus was homogenized in 0.5% Nonidet P40, 10 mM Na-EDTA, 1 mM PMSF, 1 mM iodoacetamide, 50 μ M tosylamide-2-phenyl-ethyltrichloromethyl ketone (TPCK), 10 mM Na-HEPES (pH 7.5). Washed nuclear pellet was then extracted with 2% (w/v) CCl_3COOH at 4°C. The extract was made successively 10, 15 and 25% in CCl_3COOH at 4°C, with pelleting by centrifugation of precipitates formed at each increase in CCl_3COOH concentration. The pellet obtained after addition of 25% CCl_3COOH was washed with acetone, dried, dissolved in water and thereafter fractionated by electrophoresis on a preparative-scale acetic acid-urea polyacrylamide gel [16]. The band of HMGl7 was electroeluted from the gel; the eluate was made 5 M in urea (ultra-pure, BioRad) and dialysed extensively against 1 mM Na-EDTA, 1 mM Na-HEPES (pH 7.5) at 4°C. Sucrose was added to 10% (w/v) and the sample was frozen at -70°C in multiple aliquots. The protein concentration was determined by the BioRad Protein Assay using bovine serum albumin as a standard.

HMGl7 thus obtained was at least 95% pure as judged from Coomassie-stained patterns after electrophoresis in acetic acid-urea [16], Triton-acetic acid-urea [17] and SDS [18] gels. Electrophoresis in an SDS-gel of HMGl7 iodinated with Bolton-Hunter reagent [19] showed less than 1% of the label in contaminating protein bands (unpublished data).

Two-dimensional fractionation of mononucleosomes

Peak mononucleosome fractions from sucrose gradients (Fig. 1A) were electrophoresed in the first (deoxyribonucleo-

protein, DNP) dimension at 4°C through 5% polyacrylamide gels (1.2 mm thick, 30 cm long; acrylamide: bisacrylamide w/w ratio of 27:1) which contained 0.5 mM Na-EGTA, 1 mM Na-EDTA, 10 mM Na-HEPES (pH 7.5) as described previously [10,18,20]. Second-dimension electrophoresis of double-stranded DNA was carried out in 9% polyacrylamide-SDS gels as previously described [18] except that the first-dimension gel strip was incubated in 1% SDS, 0.005% bromophenol blue, 1 mM Na-EDTA, 5 mM Tris-HCl (pH 8.0) for 3 hours at 45°C before the second-dimension electrophoresis and autofluorography of ³H-DNA.

In some of the experiments mononucleosomal DNA was electrophoresed in a single-stranded form essentially as described previously [21]. Briefly, the first-dimension 5% polyacrylamide strip was incubated for 1 hour at 45°C in 8 M urea (ultra-pure, Bio-Rad), 2 mM Na-EDTA, 89 mM Tris-borate (pH 8.3) in the presence of bromophenol blue and xylene cyanol as mobility markers. The strip was then boiled in the same buffer for 20 minutes followed by an immediate application onto a second-dimension polyacrylamide gel (8% acrylamide, 0.4% bisacrylamide; 1.5 mm thick, 16 cm long) containing 8.3 M urea, 2 mM Na-EDTA, 89 mM Tris-borate (pH 8.3). Electrophoresis was carried out at 500 V initially to quickly heat up the gel followed by 300 V until the xylene cyanol band had neared the bottom of the gel.

HMG17-monomucleosome binding assay

Equal volumes of an appropriately diluted mononucleosome sample (a single peak fraction from a sucrose gradient; see Fig. 1A) and an HMG17 sample were mixed in 0.5 mM Na-EGTA, 1 mM Na-EDTA, 10 mM Na-HEPES (pH 7.5) followed by incubation at 0°C for 20 minutes and electrophoresis of the mixture in the first (DNP) dimension as described above. In experiments described below only mononucleosomes produced from 0.35 M NaCl-washed chromatin were used. In some of the experiments both mixing and fractionation of HMG-monomucleosome samples were carried out at a higher ionic strength (40 mM NaCl was added to both the binding and first-dimension electrophoretic buffers). No attempts have been made to ascertain what fraction of added HMG17 actually binds to mononucleosomes

under conditions used. Therefore input HMG/DNA ratios given in the legend to Fig. 2 do not necessarily coincide with actual binding ratios.

RESULTS

Core Mononucleosomes containing or lacking uH2A semihistone do not differ significantly in their affinity for HMG17 in vitro

The interaction of HMG17 with core mononucleosomes was monitored by a two-dimensional (DNP+DNA) binding assay. Core (MN1) mononucleosomes that have bound either one or two HMG17 molecules per particle are initially separated from HMG-lacking MN1 mononucleosomes in the DNP dimension by low ionic strength gel electrophoresis [1-3,10,18,20]. Thereafter a second-dimension (DNA) electrophoresis of the mononucleosomes separated in the first dimension is carried out to compare DNA size distributions between HMG-containing and HMG-lacking sets of mononucleosomes.

We have shown previously that the low ionic strength (DNP) electrophoresis resolves MN1 mononucleosomes containing zero, one and two uH2A molecules per particle [10,18; see also Figs. 1 and 2]. Thus the use of the two-dimensional binding assay allows, for the first time, a direct comparison of relative affinities of HMG17 for ubiquitinated versus non-ubiquitinated core mononucleosomes, as well as monitoring of HMG-monomonucleosome binding as a function of the mononucleosomal DNA fragment size.

HeLa chromatin was washed with 0.35 M NaCl to remove most of HMG14/17 proteins and a significant proportion of histone H1 [22] followed by digestion with staphylococcal nuclease and purification of the mononucleosomes by sucrose gradient sedimentation (Fig. 1A). Most of the HeLa mononucleosomes obtained under these conditions are the [~146-bp] core (MN1) mononucleosomes, with the rest of the mononucleosomal material being the so-called "whiskers" [10,19,20], that is, mononucleosomes which contain DNA fragments slightly larger than core-size (Figs. 1 and 2; see the legend to Fig. 1 and refs. 10,18 and 20 for mononucleosome terminology). Core size is operationally defined by the single DNA spot noted on two-

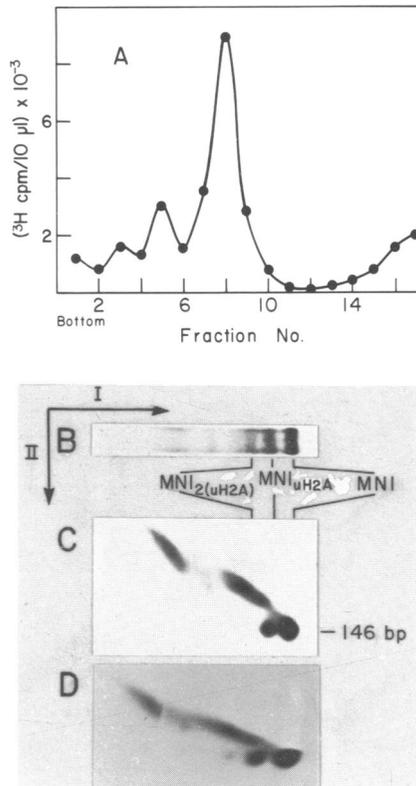


Fig. 1. Purification of HeLa mononucleosomes.

Staphylococcal nuclease digest of ^3H -thymidine-labeled HeLa chromatin (extracted with 0.35 M NaCl before nuclease digestion) was centrifuged in a sucrose gradient as described in Methods.

(A) Sedimentation profile; fraction No. 8 was used in experiments shown below; fractions No. 7 and 9 gave similar results.

(B) Mononucleosomes (fraction No. 8) were electrophoresed in the first (DNP) dimension as described in Methods; only mononucleosomal area of the gel is shown.

(C) Second-dimension (double-stranded DNA) pattern of the mononucleosomes resolved in the first (DNP) dimension.

(D) Same as in C but second-dimension (DNA) electrophoresis was carried out under denaturing conditions (see Methods).

Mononucleosome terminology: MN1: core mononucleosome containing \sim 146-bp DNA fragment and core histone octamer but lacking histone H1, HMG proteins and uH2A. MN1_{uH2A} : same but with one molecule of uH2A substituting for one molecule of H2A. $\text{MN1}_2(\text{uH2A})$: same but with both H2A molecules substituted by two molecules of uH2A (see refs.10, 18)

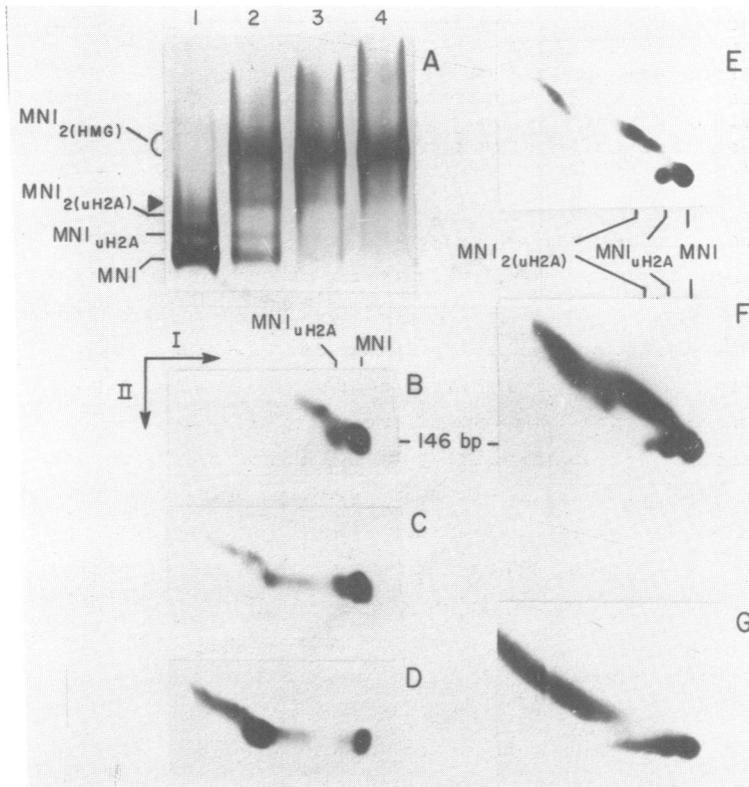


Fig. 2. Affinity of HMG17 for a mononucleosome is not influenced significantly by the presence of uH2A but strongly depends on DNA fragment size.

(A) First-dimension (DNP) patterns of the initial mononucleosome sample (A1) and of the same sample mixed with increasing amounts of HMG17 and electrophoresed under low ionic strength conditions (A2-A4) (see Methods). Input HMG17/DNA w/w ratios of 0.36, 0.54, and 0.72 in A2-A4, respectively.

(B) Second-dimension (DNA) pattern of the initial mononucleosome sample shown in A1.

(C) Same as in B but mixed with HMG17 (input HMG17/DNA w/w of 0.27).

(D) Same as in C but higher amount of HMG17 (input HMG17/DNA w/w ratio of 0.4).

(E) Same as in B but a different mononucleosome preparation (same as the one shown in Fig. 1C).

(F) Same as in E but longer fluorographic exposure to see the MN1₂(uH2A) DNA spot.

(G) Same as in F but mixed with HMG17 (input HMG17/DNA w/w ratio of 0.14).

Note a striking preference for HMG17 binding to

"whisker" mononucleosomes and the absence of significant differences in relative affinities of HMG17 to ubiquitinated versus non-ubiquitinated core (MN1) mononucleosomes.

Designations are as in Fig. 1. $MN1_2(HMG)$ denotes core mononucleosomes which have bound two HMG17 molecules per particle. An arrowhead in A denotes the position of core mononucleosomes which have bound one HMG17 molecule per particle.

dimensional (DNP+DNA) electrophoresis of the major non-H1 containing mononucleosomal particles. The appearance of discrete DNA spots ($MN1$, $MN1_{UH2A}$) on the denaturing second-dimension gel shown in Fig. 1D implies little if any variability of DNA fragment length in these preparations. Analysis by other workers have established a length of approximately 146 base pairs for the core mononucleosomal DNA fragment [6].

"Whisker" mononucleosomes lack both histone H1 and HMG14/17; they are converted upon further digestion with staphylococcal nuclease into the core (MN1) mononucleosomes [18,20,23]. "Whisker" DNP+DNA patterns display a significant internal heterogeneity (Figs. 2B and 2E; see also ref. 20). Although both relative abundance and appearance of "whisker" DNP+DNA patterns are a function of the time and conditions of nuclease digestion [18,20], a persistent result is that of one (Fig. 2B) or two (Fig. 2E) DNA "subspots" present within a continuous "whisker" DNA pattern.

Only traces of larger, histone H1-containing mononucleosomes [$MN2$; see refs. 9,10,18,20] were present in the mononucleosome preparations used in this work (see Methods).

With addition of small amounts of the purified HMG17 to the mononucleosome preparation, a new discrete DNP band corresponding to a mononucleosome containing two HMG17 molecules per particle is observed after first-dimension (DNP) electrophoresis (Figs. 2A2-2A4; cf Fig. 2A1). There is also a smear extending down from the $MN1_2(HMG)$ band halfway to the MN1 band position (Fig. 2A). Particles migrating at the lower end of this smear (denoted by an arrowhead in Fig. 2A) correspond to the core (MN1) mononucleosomes containing one HMG17 molecule per particle as has already been described by Felsenfeld, Olins and their colleagues [1,2]. In our

experiments a significant cooperativity of HMG binding to mononucleosomes is observed even under relatively low (~ 0.01) ionic strength conditions; the result is a preferential formation of $MN1_2(HMG)$ but not of $MN1_{HMG}$ particles upon addition of substoichiometric amounts of HMG17 (Fig. 2A and data not shown).

Second-dimension (DNA) electrophoresis of the mononucleosomes resolved in the first (DNP) dimension clearly shows that HMG17 binds with comparable affinities to both uH2A-containing and uH2A-lacking core (MN1) mononucleosomes (Figs. 2A-2D); positive identification of minor core-length DNA spots as those corresponding to the $MN1_{uH2A}$ and $MN1_2(uH2A)$ mononucleosomes was described previously [10,18]. Since even a 2-fold difference in relative HMG17 affinities would be readily detectable in the patterns shown (Fig. 2), and since the presence or absence of uH2A is the only detectable difference between uH2A-containing and uH2A-lacking core mononucleosomes (both of which contain a ~ 146 -bp DNA fragment), we conclude that the ubiquitin moiety of uH2A is not involved in HMG-core nucleosome interactions under conditions used. The same conclusion was reached when both HMG17-monomucleosome binding and the DNP electrophoresis were carried out in a higher ionic strength buffer (40 mM NaCl, 1 mM Na-EDTA, 0.5 mM Na-EGTA, 10 mM Na-HEPES, pH 7.5; data not shown).

Mononucleosome-HMG17 binding is greatly enhanced upon an increase of mononucleosomal DNA fragment size by as few as 3 to 5 base pairs

An additional aspect of the patterns shown in Fig. 2 is the striking preference for binding of HMG17 to mononucleosomes which contain DNA fragments even slightly (a few bp) longer than the core-length DNA (Figs. 2B-2G). These mononucleosomes ("whiskers"; see above) upon further digestion with staphylococcal nuclease can be converted into the core mononucleosomes [18,20,23].

Not only is there a striking preference for HMG17 binding to the "whisker" mononucleosomes (Figs. 2C and 2G; cf Figs. 2B and 2F, respectively), but in addition, the affinity of HMG17

for a "whisker" with a relatively large (155-165 bp) DNA fragment is comparable to the HMG17 affinity for a "whisker" whose DNA fragment size is only marginally (3-5 bp) greater than the core length (Figs. 2E-2G, and fluorographic under-exposures of Fig 2G (not shown)). If the binding of HMG17 to a "whisker" mononucleosome was significantly enhanced upon a further increase of the "whisker" DNA fragment size, one would expect HMG17 to bind preferentially to larger "whisker" mononucleosomes. As a result a gradient of fluorographic intensity would form along the second-dimension (DNA) image of displaced (HMG-bound) "whiskers"; this is not observed (Fig. 2G and data not shown).

Furthermore, the enhanced binding of HMG17 is also observed for small whisker mononucleosomes migrating to the right of MN1_{uH2A} (Figs. 2F and 2G). These whisker mononucleosomes are devoid of uH2A and other proteins not found in MN1 mononucleosomes [10]. Thus the presence of uH2A is not required for the enhanced binding of HMG17 by the whisker mononucleosomes.

Second-dimension electrophoresis of the mononucleosomal DNA under denaturing conditions shows that neither "whisker" nor core DNA fragments have any significant amounts of internal single-stranded nicks or gaps (Fig. 1D; cf Fig. 1C). The presence of short protruding stretches of single-stranded DNA at the ends of these DNA fragments is not excluded, however [22].

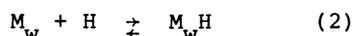
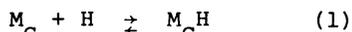
Lastly, the same striking preference of HMG17 binding to slightly larger than core-length mononucleosomes is observed also with a higher ionic strength binding buffer (40 mM NaCl, 1 mM Na-EDTA, 0.5 mM Na-EGTA, 10 mM Na-HEPES, pH 7.5; data not shown).

DISCUSSION

The two major results of this work are first, that HMG17-monomonucleosome interactions in vitro are not influenced significantly by the presence of uH2A in a core mononucleosome, and second, that even a slight increase in the size of a mononucleosomal DNA fragment (by as few as 3 to 5 bp

over the core length) leads to a striking enhancement of HMGl7-monomucleosome interactions (see Results).

To calculate the approximate, "order-of-magnitude" ratio of apparent affinities of HMGl7 for "whisker" versus core mononucleosomes we approximate the HMGl7-monomucleosome interactions as follows:



in which M_C is the core (146-bp) mononucleosome, M_W is the "whisker" mononucleosome (see Results) and H is HMGl7 (treated as a dimer; see below).

That M_C can be treated in a first approximation as a single kind of particle is supported by our finding (see Results) that HMGl7 binds with close affinities to both ubiquitinated and non-ubiquitinated core mononucleosomes. Other data (see Results) indicate that HMGl7 binds with comparable affinities to both larger and smaller of the "whisker" mononucleosomes, thus justifying the treatment of M_W in a first approximation as a single kind of particle as well. An additional simplification is to ignore any incomplete cooperativity of HMGl7 binding in our system; as can be readily shown, this assumption does not significantly alter the parameter being calculated.

Equilibrium association constants for reactions (1) and (2) are

$$K_C = \frac{[M_C H]}{[M_C][H]} \quad (3) \quad \text{and} \quad K_W = \frac{[M_W H]}{[M_W][H]} \quad (4)$$

The ratio K_W/K_C (to be calculated) does not depend on [H] and is

$$\frac{K_W}{K_C} = \frac{[M_W H][M_C]}{[M_C H][M_W]} \quad (5)$$

Turning now to a concrete case (Figs. 2B-2C), where a substoichiometric amount of HMGl7 is added to the initial mononucleosome sample (Fig. 2B) to give the pattern shown in Fig. 2C, we note from direct comparisons of relative fluorographic intensities in the areas corresponding to M_C , M_W , $M_C H$ and $M_W H$ in Fig. 2C that for this particular point in the interaction diagram:

$$\frac{[M_C]}{[M_H]} = 10 \quad (6) \quad \text{and} \quad \frac{[M_{WH}]}{[M_W]} \geq 50 \quad (7)$$

Both (6) and (7) are minimal estimates. Higher fluorographic exposures were necessary to be sure that the ratio $(M_{WH})/(M_W)$ is at least 50 and may be much more (data not shown). Substituting (6) and (7) into (5) gives an approximate, lower-limit estimate of the ratio of HMG17 affinities (equilibrium association constants) for "whiskers" versus core mononucleosomes:

$$\frac{K_W}{K_C} \geq 500 \quad (8)$$

Thus HMG17 binds to a mononucleosome containing as few as 3 to 5 bp of "extra" (linker) DNA at least 500 times more tightly than to the core mononucleosome. An analogous type of gel analysis has been carried out by Fried and Crothers for the lac repressor-lac operator system [24].

One interpretation of this result is that the in vivo HMG17 binding site on the nucleosome includes a few bp of linker DNA, in agreement with earlier suggestions [1-3]. The striking magnitude of the observed effect, however, leads us to suggest that the previously detected preferential in vitro binding of HMG14/17 to mononucleosomes derived from certain active genes [reviewed in ref. 6] can be explained if these staphylococcal nuclease-produced mononucleosomes have on average slightly (3 to 5 bp) larger than the core-length DNA fragments, possibly also (but not necessarily so) with staggered (partially single-stranded) DNA ends. The latter feature, if present, should additionally stabilize HMG-nucleosome complexes since HMG17 is known to bind more tightly to single-stranded than to double-stranded DNA [25].

To our knowledge, the literature data on the DNA fragment size distribution of "active" versus bulk mononucleosomes are compatible with the above hypothesis. Indeed, an earlier in vitro study by Albright et al. [3] using an approach less sensitive to small changes in mononucleosomal DNA length than the one used above (Fig. 2), did report a longer average DNA length of nucleosomes reconstituted with HMG14/17. No suggestion on the possible relevance of this phenomenon to the

"active" nucleosome-HMG puzzle was made, however. In another recent work, Weisbrod [5] has compared properties of bulk chicken erythrocyte mononucleosomes with those of the mononucleosomes that were preferentially retained on the HMG affinity column. Although the author's conclusion is that there are no significant differences between DNA fragment sizes of the bulk versus retained mononucleosomes, examination of the actual data [Fig. 7 in ref. 5] suggests a small but detectable (approximately 5 bp) difference between the average DNA lengths of the retained and bulk mononucleosomal fractions. Given a striking ($\sim 10^3$ -fold) enhancement of HMG17 binding to a mononucleosome upon an increase of its DNA fragment size by as few as 3 to 5 bp (see Results), the mononucleosomal DNA patterns observed by Weisbrod [5] are formally compatible with our hypothesis.

Since the presence of HMG14 or 17 on the nucleosomal array during digestion by staphylococcal nuclease partially protects the linker DNA from "nibbling" by the nuclease [3,8,18,22], our hypothesis appears to be sufficient to explain the phenomenon of preferential in vitro HMG binding to mononucleosomes which contained HMGs at the time of their excision from chromatin by staphylococcal nuclease.

A related problem of why and how HMG14/17 become preferentially associated with at least some of the active genes in vivo is not addressed by our experiments.

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