HMG 14/17 binding affinities and DNAase I sensitivities of nucleoprotein particles

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Received 15 May 1983; Revised and Accepted 12 September 1983

ABSTRACT

We show that ordinary (bulk) chicken erythrocyte nucleosomes are digested more rapidly by DNAase I when they are associated with high mobility group (HMG) proteins 14/17. Digestion of HMG 14/17-nucleosome complexes, under conditions where the DNA in control nucleosomes is digested to 10 to 20% acid solubility, results in a particular depletion of single-strand DNA fragments greater than 80 nucleotides in length, relative to the DNA fragments produced from control nucleosomes. Additionally, we show that staphylococcal nuclease digests of H1/H5-depleted chromatin contain an abundant subclass of nucleosomes that are not present in appreciable amounts in digests of native chromatin. These nucleosomes contain longer lengths of DNA and have lower electrophoretic mobilities than core particles. HMG 14/17 associates highly preferentially with these nucleosomes and renders them sensitive to DNAase I, similar to what has been found for active nucleosomes.

INTRODUCTION

Active genes appear to be packaged into nucleosomes that are distinguishable from those of inactive chromatin (1,2). In chick red blood cells, purified mononucleosomes from active chromatin, although apparently similar to bulk nucleosomes, are preferentially recognized by the nonhistone chromosomal proteins HMG 14 and HMG 17 (3,4). Moreover, this interaction makes active nucleosomes especially sensitive to DNAase I (3). Despite intensive study, the distinguishing structural or chemical features of active nucleosomes and the manner whereby they are rendered DNAase I sensitive remains obscure.

Some properties which appear to be characteristic of active nucleosomes have been found. Albanese and Weintraub (5) observed that chick erythrocyte nucleosomes that are enriched in globin gene DNA sequences migrate more slowly than core particles on polyacrylamide gels. Such nucleoprotein particles contained longer than core particle length DNA and were associated with HMG 14 and 17. These authors showed that the low particle mobility was not due to the association of these nucleosomes with H1 or H5, and they provided evidence that it could not be attributed completely to the presence of HMG proteins, or to

the increased DNA length. In order to explain their observations they postulated the existence of alterations of an unknown nature in the nucleoprotein particle. Recently, Weisbrod (6) purified particles that were enriched in active nucleosomes by HMG 14/17-affinity chromatography, and clearly showed that these particles exhibit low electrophoretic mobilities in the absence of HMG proteins. He also concluded that these particles must be altered in some way.

The interaction between HMG 14 or 17 and bulk nucleosomes (not containing specific DNA sequences) has also been studied (4,7-9). Bulk nucleosome core particles appear to specifically bind two HMG 14 or 17 molecules per particle (4,7-9). The major sites of interaction are located near the ends of the DNA (4,7). No substantial structural change in the nucleosome occurs upon binding the HMG proteins (4,9). Also, no HMG-induced DNAase I sensitivity was observed in these studies (4,7), in contrast with what has been found for active nucleosomes, using different techniques (1,3). This finding suggested that active nucleosomes must, in some way, be quite different from bulk nucleosomes.

Very recently, Swerdlow and Varshavsky (10) made the observation that bulk nucleosomes containing longer than core particle length DNA have a strikingly enhanced affinity for HMG 14/17. These authors found that nucleosomes, containing as few as 3 to 5 base pairs of DNA in excess of the length contained in core particles (146 bp), possessed an association constant that was more than two orders of magnitude higher than that for HMG-core particle binding. They pointed out that this affinity difference was sufficient to account for the preferential in vitro binding of HMG 14/17 to active mononucleosomes, which may contain increased DNA lengths (5,6). In contrast with the studies cited above, this study suggested that active mononucleosomes may not be very different from ordinary nucleosomes; the active mononucleosomes may simply contain slightly longer lengths of DNA (10). The problem of how HMG 14/17 induces DNAase I sensitivity in active nucleosomes and chromatin was not addressed by these authors. Thus it is not at all clear to what extent active nucleosomes differ from ordinary nucleosomes and how HMG 14/17 induces DNAase I sensitivity in active nucleosomes.

In this paper we report two new observations which bear on the problems described above. First, we show that, when all of the DNA is examined, it is clear that HMG 14/17 induces DNAase I sensitivity in bulk nucleosomes and core particles. Second, we show that when bulk chromatin fragments are stripped of H1 and H5 by a method which does not disturb the internucleosome

spacing, micrococcal nuclease digestion produces an abundant subclass of nucleosomes which bind HMG 14/17 molecules with very high preference. We discuss how purified active mono-nucleosomes and chromatin might acquire the properties of preferential HMG binding and HMG-induced DNAase I sensitivity.

MATERIALS AND METHODS

Preparative procedures

Salt-washed (H1/H5-free) chicken erythrocyte nucleosomes containing 145 to 165 base pairs of DNA were prepared as previously described (11), except that staphylococcal nuclease digests of nuclei were fractionated at room temperature on a Biogel A 5-m column in 0.6 M NaCl, 10mM Tris-HCl, pH 8.0, 1 mM EDTA. The monomer fraction was dialyzed against 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and was concentrated by ultrafiltration (Amicon YM10 membrane). Core particles were prepared from a preparation of nucleosomes that were not salt washed, as described by Simpson (12). H1/H5-stripped chromatin was prepared by treating chromatin with Bio-Rad AG 50W-X2 resin a 0.35M NaCl containing buffer, as previously described (13). "Long nucleosomes" from H1/H5-stripped chromatin were purified on isokinetic sucrose gradients (see ref. 12) in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA in a SW41 rotor. HMG 14/17 was prepared from 0.35 M NaCl, 5 mM potassium phosphate, pH 7.0 washes of chicken erythrocyte nuclei by the trichloroacetic acid method described by Sandeen et al. (4). Sodium dodecyl sulfate gel analysis (as performed in Ref. 12) indicated that our preparations were slightly enriched in HMG 14, as judged by staining with Coomassie blue. To prepare nucleosome (or core particle)-HMG 14/17 complexes, the components were mixed directly in 0.1 M NaCl, 20 mM Tris-HCl, pH 7.2. These samples were either diluted two-fold with 2X sample buffer for electrophoresis, or were dialyzed against 0.01 M Tris-HCl, pH 7.4, 0.01 M NaCl, 5mM MgCl₂, the (RSB) buffer used by Weisbrod et al. (3), for DNAase I digestion.

Analysis of DNA and nucleoprotein particles

Nucleoprotein particles were separated by electrophoresis on 4% polyacrylamide gels in 0.178 M Tris, 0.178 M boric acid, and 5 mM Na₂ EDTA (2 x TBE), as described by Sandeen et al. (4). Nondenatured DNA fragments were separated on 5% polyacrylamide gels in TBE buffer. Single strand DNA fragments were separated by electrophoresis on 10% or 12% polyacrylamide gels in TBE buffer containing 7 M urea, as previously described (11). Nuclease digestion reactions were stopped at the stated times by adjusting measured portions, withdrawn from a sample, to 10 to 20 mM Na₂ EDTA. DNA was

extracted and prepared for electrophoresis as described previously (14), or as stated. For quantitative analysis of single strand DNA fragment distributions, denaturing gels were stained overnight in 0.005% Stains-All (Bio-Rad) in 50% formamide and destained in running water; the stained gel was scanned with an E-C Instruments Co. densitometer. Otherwise, DNA gels were stained with ethidium bromide and photographed under UV illumination, as previously described (14). DNA was eluted from polyacrylamide gels as described by Maxam and Gilbert (15). For measurement of the extent of DNAase I digestion, aliquots containing about 0.5 mg/ml DNA were adjusted to 10 mM EDTA, pH 8.0, 0.1% SDS and 0.2 mg/ml Proteinase K (Boehringer Mannheim), incubated 2 hr at 37°, adjusted to 1% SDS, heated 2 min at 100°, chilled and adjusted to 0.5 M NaCl, 0.5 M perchloric acid, 0.1 mg/ml bovine serum albumin (Miles). The fraction that was soluble was determined from the absorbance at 260 nm of the supernatant fluids, diluted to 0.5 ml with 0.5 M NaCl, 0.5 M perchloric acid, and the pellets, dissolved in 0.1 N NaOH.

RESULTS

HMG 14/17 induces DNAase I sensitivity in bulk nucleosomes

In previous studies (4,7), the relative DNAase I sensitivities of core particles and HMG 14/17-core particle complexes were assessed by gel electrophoresis of DNA from core particles with end-labeled DNA. For core particles reconstituted from polyd(A-T) and core histones, Mardian et al. (7) found that the presence of bound HMG 14 did not greatly alter the overall rate of DNAase I digestion. In contrast with this result, Sandeen et al. (4) found that the rate of digestion of HMG-nucleosome complexes was considerably slower than that of nucleosome cores alone. Although the rate of DNAase I digestion of end-labeled nucleosome DNA to acid soluble fragments is only slightly faster than that of total nucleosome DNA (16), the overall relative digestion rates ± HMG proteins may not be accurately reflected by the electrophoretic behavior of only the end-labeled fragments. This is because there are particularly susceptible sites near the labeled DNA ends (16,17), which become protected by bound HMG proteins (4,7). Thus, in uncomplexed control core particles the labeled DNA ends might be cut off rather easily, making the remaining DNA fragment undetectable on gels. Protection of these preferred DNAase I cutting sites by HMG proteins might make HMG-core particle complexes appear to be more resistant than the control particles, even though the total DNA might be digested away more rapidly. Because of these considerations, we have re-examined the overall rate of DNAase I digestion for

Figure 1. DNAase I digestion of nucleosomes and nucleosome-HMG 14/17 complexes under identical conditions. (A) Kinetics of digestion. Salt-washed nucleosomes and HMG-nucleosome complexes were digested with 6 units/ml DNAase I at 37° for the times indicated, and the fractions of the original samples that were soluble in perchloric acid was determined. (B) Densitometer scans of a 10% polyacrylamide gel containing 7 M urea. Nucleosome core particles (a-c) and HMG-core particle complexes (d-f), each at about 12 A₂₆₀ units/ml were digested with 0.75 units/ml DNAase I at 37° for 5, 10 and 20 min. Samples were prepared for electrophoresis without loss of DNA, as described in the text. The arrow marks the "80" nucleotide single-strand DNA fragment. The 30 nucleotide fragment at the far right of each scan (shown truncated) ran as a large peak that was sharpened owing to its co-migration with the ion front.

nucleosomes complexed with HMG 14/17, compared to control nucleosomes, digested under identical conditions.

HMG-nucleosome complexes, containing approximately two HMG proteins per nucleosome (see below), were dialyzed against RSB buffer (see Materials and Methods), along with a control sample containing nucleosomes and buffer. The dialyzed samples were next adjusted to the same DNA concentration and volume and were digested at the same concentration of DNAase I for increasing times. In this experiment we used 0.6M NaCl-washed nucleosomes to insure that H1, H5

and non-histone proteins were absent. Fig 1A shows that there was a significant increase in the digestion rate of the nucleosomes upon complexing with HMG 14/17. The increased amount of acid soluble DNA produced, at each time point, due to HMG binding reflects an increase in the rate of digestion, rather than an increase in the maximal extent of digestion possible, since DNA in the control core particles and chromatin can be digested to greater than 95% acid solubility by DNAase I (18 and unpublished observations). Also, we note that both samples were insoluble in the Mg^{++} -containing digestion buffer.

Next, we compared the distributions of single-strand DNA fragments arising from mild DNAase I digestions of HMG 14/17-core particle complexes and of control core particles. These nucleosome core particles contained 145 base pairs of DNA and were not exposed to salt concentrations greater than 0.1M. After RSB dialysis the two samples were adjusted to the same volume and digested, under the same conditions, with DNAase I for increasing times. The total digests were then incubated with Proteinase K, adjusted to 1% SDS, heated and loaded on the gel. Fig 1B shows that in each case fragments that are multiples of about 10 nucleotides were produced. Moreover, the total integrated intensities of the scans reflect the increased rate of digestion of HMG-nucleosome complexes at low extents of digestion (10, 14 and 18% acid soluble in a, b and c, respectively), consistent with Fig. 1A. In control core particles, DNA fragments with lengths greater than 30 nucleotides were depleted only 10% in the 20 min digest (c) compared to the mild 5 min digest (a). In contrast, DNA fragments greater than 30 nucleotides long were depleted 55% in the 20 min digest (f) compared to the 5 min digest (d) for HMG-core particle complexes. Additionally, Fig 1B shows that the 20 min DNAase I digestion of HMG-core particle complexes (18% acid soluble DNA in the control, c) resulted in a particular depletion of the larger single strand fragments. For example, the depletion of DNA fragments longer than 70 nucleotides from the HMG-core particle complexes was 3 times that from the control core particles, and the depletion of fragments longer than 90 nucleotides was 4 times that from the control particles.

Nucleosomes from H1/H5-stripped chromatin have a greatly increased affinity for HMG 14/17

We next show that nucleosomes that preferentially bind HMG 14/17 are produced in abundance from chromatin stripped of H1 and H5. First, we describe some of the characteristics of nucleosomes from H1/H5-stripped chicken erythrocyte chromatin.

Figure 2. Staphylococcal nuclease digestions of chromatin samples analyzed on 5% polyacrylamide gels. Lanes s show Hinc II digests of ϕ X174 RF DNA. (A) DNA fragments from native chromatin digested in 20 mM Tris-HCl, pH 7.2, 0.2 mM Na_2EDTA , 0.6 mM CaCl_2 at 37° with 0.12 units enzyme per μg DNA for 0.5, 1, 2, 4 and 8 min, lanes 1-4. Lane u is an undigested sample. (B) DNA fragments, lanes 1-4, and corresponding nucleoprotein, lanes 5-8, from H1/H5-depleted chromatin digested under the same conditions as in (A), but with 0.06 units enzyme per μg DNA for 0.5, 1, 2 and 4 min. The arrow indicates the position of the slowly migrating nucleoprotein monomer.

Figure 3. Analysis on 5% polyacrylamide gels of mononucleosomes produced from H1/H5-stripped chromatin by staphylococcal nuclease. (A) DNA was eluted from faster (L) and slower (U) migrating nucleoprotein monomers. Lane S shows a Hinc II digest of 0X 174 RF DNA. (B) Purified 11S particles were digested with 0.08 units BAL-31 exonuclease per μ g DNA (20 units/ml) in 20 mM Tris-HCl, pH 7.2, 0.2 mM Na₂EDTA, 5 mM CaCl₂, 5 mM MgCl₂ at 25° for 0, 5, 10, 20 and 60 min. Lanes 1-5 show the trimming of DNA, while lanes 6-10 show the corresponding nucleoprotein particles. This gel was run for a longer time than the one shown in Fig 2B to achieve a larger separation of monomer nucleoprotein particles; only the lower portion of the gel is shown.

When chromatin is stripped of H1 (and H5) by gentle methods that do not disturb the internucleosome spacing, a new and rather unexpected class of mono-nucleosomes is produced in abundance upon staphylococcal nuclease digestion (14). This is illustrated in Fig. 2. Fig 2A shows typical staphylococcal nuclease digestions of native (H1/H5-containing) chicken erythrocyte chromatin. The amount of mononucleosomes produced containing DNA longer than 200 base pairs is small. As digestion becomes more extensive, 165 base pair chromatosomes (12) and 145 base pair core particles accumulate. In contrast, Fig 2B (lanes 1-4) show that a substantial amount of DNA with lengths broadly distributed around 210 base pairs is produced from H1/H5-stripped chromatin. The faster migrating mononucleosome DNA is much more homogeneous and has a length of approximately 165 base pairs, at the lower extents of digestion. Lanes 5-8 show, for the same digests, that a discrete subclass of slower migrating monomer nucleoprotein particles (arrow) is produced, which persists until almost all of the chromatin is digested to

monomers. We have obtained very similar results using a wide range of enzyme concentrations, enzyme to substrate ratios, and digestion times.

Fig 2B suggests that the slower migrating monomer nucleoprotein particles contain longer lengths of DNA than the faster migrating monomer particles. We confirmed this by eluting the DNA from the upper and lower nucleoprotein monomer bands. The particles migrated similar to that shown in Fig. 2B, lane 7; the arrow denotes the position of the upper band. Fig 3A shows that the slower moving particles do contain longer DNA. Moreover, trimming the tails from sucrose gradient-purified mononucleosomes produced from H1/H5-stripped chromatin, with BAL-31 exonuclease results in only fast migrating nucleoprotein particles which contain predominantly 145 base pair DNA (Fig 3B). In contrast, Exonuclease III treatment of these particles did not substantially alter the particle mobility (not shown). The fast migrating DNA fragment in lane 5 is about 120 base pairs long, and results from invasion by the exonuclease into the core particle, which occurs upon extensive digestion (unpublished observations). Also, fast and slow migrating particles co-sedimented at 11 S as a single symmetrical peak (not shown). These data indicate that the low electrophoretic mobility of the "long nucleosomes" is dependent upon the increased DNA length contained. This behavior must be the result of either a shape change or an alteration of the particle involving a increase in the effective charge (5). In either case, the magnitude of the effect appears substantial.

Because these "long nucleosomes" from H1/H5-depleted chromatin may share at least one property with some active nucleosomes, a low electrophoretic mobility (5), we examined the binding of HMG 14/17 to these particles. HMG 14/17-core particle complexes have a substantially lower electrophoretic mobility than core particles, providing a convenient binding assay (8,7,4). Fig 4A shows the titration of core particles with HMG 14/17. A discrete slower moving band appears upon addition of increasing amounts of the HMG proteins, and increases in intensity until most of the particles migrate to the position of this band (lane 4). At higher levels of HMG 14/17 the core particles continue to bind these proteins and migrate in a diffuse fashion, as observed by others (7,4).

We next titrated purified mononucleosomes, produced from H1/H5-depleted chromatin, with HMG 14/17 (Fig 4B). When smaller amounts of the same HMG preparation used in A were added to these nucleosomes, the upper band in lane 1 selectively decreased in intensity, until it disappeared (lanes 3,4); this nucleoprotein migrated rather diffusely with a lower mobility. The intensity

Figure 4. Electrophoresis on 4% polyacrylamide gels showing the titration of nucleoprotein monomers with HMG 14/17. (A) 5 μ g of core particles (amount of DNA) for each lane, were mixed with 0, 1, 2, 6 and 12 μ l of a solution of HMG 14/17 lanes 1-5, respectively. The amount of HMG 14/17 used in lane 4 corresponds to about 2 moles per mole of core particles. (B) 5 μ g of purified 11 S particles from H1/H5-stripped chromatin (amount of DNA) for each lane were mixed with 0, 0.5, 1, 2 and 4 μ l of the same HMG 14/17 solution used in (A). Arrows mark the position of the uncomplexed slowly migrating particles. (C). Densitometer scans of portions of lane 1 (solid curve) and lane 4 (broken curve) from the negative of the photograph shown in (B). The same densitometer settings were used for each scan, and the scans were aligned based upon distance from the origin of electrophoresis. S denotes the initial position of the slowly migrating monomer particle (lane 1), and F denoted the position of the faster migrating monomer particle.

Figure 5. Electrophoresis of single-strand DNA fragments on a 12% polyacrylamide gel containing 7 M urea. Slowly migrating 11 S particles from H1/H5-depleted chromatin were selectively associated with HMG 14/17 as in Fig 4B, lane 3, and the sample was digested at 37° with 0.05 units/ml DNAase I for 0, 10, 30 min (d-f). Digestion, under identical conditions, of a control sample not associated with HMG 14/17 is shown in (a-c). The gel was stained with ethidium bromide; densitometer scans of the photographic negative are shown.

of the lower band remained unchanged. A scan of lane 4 (broken curve) is shown superimposed upon lane 1 (solid curve) in Fig 4C; the selective interaction of the slowly migrating particles (labeled S) is evident. Fig. 4B and C indicates that the slowly migrating nucleosome-like particles interacted with HMG 14/17 with a high preference. An analysis of band intensities and a simple calculation (not included) indicate that the slowly migrating particles must have an association constant about two orders of magnitude greater than that of the 165 base pair particles. Upon further addition of HMG 14/17, the faster moving band also decreased in intensity with the appearance of a discrete band similar to that of the HMG-core particle complex, as in A (lane 5).

To determine whether the slow moving nucleosome-like particles also exhibit HMG 14/17 induced DNAase I sensitivity, we selectively added HMG 14/17 to these particles as in Fig 4B, lane 3. We have not been able to separate the long nucleosomes from the shorter nucleosomes in the sample. The mixture of particles was digested lightly with DNAase I along with a con-

Figure 6. Electrophoresis of single-strand DNA fragments on a 10% polyacrylamide gel containing 7 M urea. Lanes 1-4, high molecular weight chromatin digested at 37° with 0.5 units/ml DNAase I for 0, 2, 5 and 10 min. Lanes 5-8, a portion of the same chromatin sample, associated with HMG 14/17, digested under identical conditions.

trol sample not containing HMG proteins, as described above. Typical digestion series are shown in Fig. 5. The HMG-induced DNAase I sensitivity is apparent from the band intensities relative to those of the control sample, digested at identical conditions, even though only approximately half of the particles were associated with HMG proteins. In the absence of HMG 14/17 (a-c) some of the longer nucleosomes and the small amount of nucleosome oligomers appear to be converted to core particles before internal cutting by DNAase I becomes appreciable. Thus, the 140-160 nucleotide fragment bands initially increase in intensity (b compared with a). In the presence of HMG 14/17 (d-f), the longer nucleosomes, which are selectively complexed with the HMG proteins, appear to be digested to small fragments directly. Analysis of the DNA fragment patterns shown in Fig 5 (and in many similar experiments, not shown) indicate that an approximately two-fold overall (including the uncomplexed shorter nucleosomes) depletion of single-strand DNA fragments greater than 80 nucleotides long is induced by HMG 14/17 at approximately 10% acid solubility of control particles (c). Therefore, the long nucleosome-

HMG complexes in the sample must be quite DNAase I sensitive.

We also tested high molecular weight (average size of about 50 nucleosomes) H1/H5-stripped chromatin for HMG-induced DNAase I sensitivity. Fig 6 shows digestions of a control sample (lanes 1-4) along with HMG-complexed chromatin (lanes 5-8). Approximately two HMG molecules per nucleosome were added. This experiment shows that, for high molecular weight chromatin, HMG 14/17 decreases the rate of digestion by DNAase I.

DISCUSSION

We have shown that when ordinary nucleosomes are digested to about 18% acid solubility with DNAase I, HMG 14/17-nucleosome complexes are digested to nearly 30%. This difference results in a 3 to 4 fold greater depletion of the longer single-strand DNA fragments produced from HMG-nucleosome complexes than from control nucleosomes. Also, we have found that isolated nucleosomes from H1/H5-stripped chromatin are highly DNAase I sensitive when complexed with HMG 14/17. Because there is an appreciable decrease in thermal stability with DNA size in this size range (19,1), the rate or plateau level of hybridization of DNAase I produced DNA fragments to a cDNA probe should, under stringent conditions, be dominated by the larger fragments (1). Consequently, we think that the HMG 14/17-induced 3 to 4-fold depletion of single-strand DNA fragments containing globin gene sequences at 10 to 20% overall digestion, measured by hybridization kinetics (1), is consistent with the HMG-induced depletion of larger fragments observed here for bulk nucleosomes. Therefore, preferential binding of HMG 14/17 to ordinary nucleosomes containing active gene sequences should be sufficient to render these nucleosomes preferentially DNAase I sensitive. We do not know how HMG 14/17 subtly perturbs the structure of nucleoprotein particles to increase the sensitivity of DNA to DNAase I. Uberbacher et al. (9), based upon neutron scattering studies, have suggested that the increased DNAase I sensitivity may be the result of a slight unwinding of the DNA in addition to a compaction of the histone core.

The detailed analysis of core particle DNA cutting by DNAase I of Lutter (17) provides some insight into the cause of the apparent discrepancy between our results and those of Sandeen et al. (4). Lutter has shown, for core particles labeled at the 5'-ends, that when only 2.7 (average value) cuts per DNA strand occur, 50% of the label appears in DNA fragments 10 nucleotides from the ends. His samples were analyzed without ethanol precipitation of the DNA, a procedure which results in the loss of a large fraction of the 10

and 20-nucleotide fragments (20). Thus the ends of the core particle DNA are readily cut off by DNAase I, and the intensity of the 146 base pair (intact) labeled fragment would decrease markedly to about 3.5% of the initial value for the above case, according to Lutter's analysis. Since HMG 14/17 protects the DNA ends (4,7), the results of Sandeen et al. (4) are not inconsistent with a significant decrease in the abundance of the larger (146-80) total single strand DNA fragments produced by DNAase I digestion of HMG-core particle complexes (Fig 1B).

The failure of HMG 14/17 to induce DNAase I sensitivity in high molecular weight H1/H5-stripped chromatin may be a consequence of the fact that such chromatin fragments still condense to a large extent in the Mg^{++} -containing digestion buffer (21). Alternatively, HMG 14/17 may condense high molecular weight H1/H5-depleted bulk chromatin to some extent (22). Nevertheless, our findings that isolated (noninteracting) core particles, as well as nucleosomes containing approximately 70 base pairs of excess DNA, exhibit HMG-induced DNAase I sensitivity suggest that H1/H5-depleted chromatin that is not condensed (see discussion below) may well be rendered DNAase I sensitive by HMG 14/17.

The induction of DNAase I sensitivity in nucleosomes by HMG 14/17 may be specific for these proteins. For example, the proteins H1/H5, which also bind tightly and rather specifically to nucleosomes, do not induce DNAase I sensitivity (12). The rate of digestion (from acid soluble A_{260}) of chromatosomes (165 base pair nucleosomes complexed with H1 or H5) is about 3-5 times slower than that of core particles. Moreover, the rate of digestion of chromatosomes, measured by acid solubility of 5'- ^{32}P -end-labeled particles, is about 5-10 times slower than that of core particles (12).

We have also shown that HMG 14/17 binds with very high preferences to the longer nucleosomes, produced in abundance from H1/H5-depleted chromatin (23,14). Because of their abundance (approximately 70% of the monomer nucleosomes have DNA lengths broadly distributed around 210 base pairs, Fig 2B), these particles obviously, are not substantially enriched in active gene sequences. The low electrophoretic mobilities of these particles are dependent upon the increased DNA length contained. Because of its resistance to staphylococcal nuclease (Fig 2B), this excess DNA is apparently protected by regions of some of the core histones, as suggested by Weischet et al. (23) for H1-depleted calf thymus nuclei. Moreover, there is evidence that H1(H5)-free nucleosomes that contain excess DNA may have structures somewhat different from core particles (24). Thus, the properties of ordinary (bulk)

nucleosomes from H1/H5-depleted chromatin are similar to those of active nucleosomes (3,5,6), which comprise only a small fraction of the genome for particular DNA sequences. The 210 base pair DNA length associated with "long nucleosomes", however, is clearly longer than that associated with some active mononucleosome preparations (4,6).

A substantial fraction of the nucleosomes produced from H1/H5-depleted chicken erythrocyte chromatin contains excess DNA, even upon extensive staphylococcal nuclease digestion (Fig 2B). Thus, our finding that these particles preferentially bind HMG 14/17 is roughly consistent with the observations of Swerdlow and Varshavsky that nucleosomes containing excess DNA preferentially bind HMG 14/17 (10). However, there are some differences, concerning preferential HMG binding, between our observations and theirs, which could be of significance. We find, using chicken erythrocyte nucleosomes, that HMG 14/17 binds much more tightly (approximately a 100-fold difference in association constants) to nucleosomes containing approximately 210 base pairs of DNA compared to nucleosomes containing approximately 165 base pairs (see Fig 2B, 3A, 3B for size calibration). They report, using HeLa nucleosomes, that HMG 17 binds much more tightly (greater than a 100-fold difference in association constants) to nucleosomes containing 3 to 5 base pairs over the core DNA length (146 base pairs). Moreover, they find no further enhancement of the HMG 17 affinity for nucleosomes containing as large as 165 base pairs of DNA (longer lengths were not examined).

Using the same techniques reported here, we have examined the relative HMG 14/17 affinities of chicken erythrocyte mononucleosome particles containing 146 versus 165 base pairs, and have confirmed one of the observations of Swerdlow and Varshavsky with the same materials used in this study (J. Zelif and A. Stein, unpublished observations). We find that 165 base pair particles do bind HMG 14/17 significantly more tightly than 146 base pair core particles, and 210 base pairs nucleosomes bind HMG 14/17 much more tightly still. The very high affinity of 210 base pair nucleosomes, compared to core particles, suggests that nucleosomes in chromatin that are not tightly compacted into higher-order structures might have much higher affinities for HMG 14/17 than nucleosomes in highly condensed chromatin.

Swerdlow and Varshavsky (10) have proposed that nucleosomes which were associated with HMG 14/17 at the time of their excision from chromatin by staphylococcal nuclease should contain slightly increased DNA lengths as a consequence of the partial protection of linker DNA by these proteins. This hypothesis can explain how isolated mononucleosomes from active chromatin

could be recognized by HMG 14/17. However, this hypothesis does not explain how HMG 14/17 preferentially associates with the nucleosomes in active chromatin (1,3), and it appears to require that active nucleosomes must be recognizable by HMG 14/17, in the first place, through some other mechanism.

Our observations suggest a hypothesis for how HMG 14/17 may induce DNAase I sensitivity in active chromatin. There is considerable evidence that active chromatin has a less compact structure than bulk chromatin (see, for example refs. 25,26). Although some evidence to the contrary has recently appeared (27), we will assume here that active chromatin is less condensed than bulk (inactive) chromatin. Since H1 and H5 can condense nucleosome arrays (28-31,21,32-36), and the H1/H5 histones exchange in chromatin at physiological ionic strength (37), it is reasonable to assume that some features of active chromatin preclude its condensation by H1 or H5. Candidates for this role include: unusual DNA structures (38,39), histone modifications (not necessarily on all nucleosomes), see (40) and references contained therein, the presence of specific tightly-bound proteins (41,42), irregular nucleosome arrangements or nucleosome-free gaps (43-48). All of these features have been implicated in active chromatin structure. Moreover, it is plausible that the noninteracting nucleosomes in such noncondensed (active) chromatin may have properties similar to those of isolated nucleosomes from H1/H5-depleted chromatin, studied here. Thus these nucleosomes would be expected to interact very strongly with HMG 14/17 and to be thereby rendered DNAase I sensitive. The nucleosomes of active chromatin need not be inherently different from bulk nucleosomes to possess these properties.

ACKNOWLEDGEMENTS

We thank Dr. Minou Bina for helpful discussion and Ms. Camille Alexander for typing the manuscript. This work was supported by grant GM 27719 (to A. S.) from the U.S. Public Health Service.

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