Influence of nonhistone chromatin protein HMG-1 on the enzymatic digestion of purified DNA

Kamini Shastri, Paul J. Isackson, James L. Fishback, M. Daniel Land and Gerald R. Reeck*

Department of Biochemistry, Kansas State University, Manhattan, KS 66506, USA

Received 24 May 1982; Revised and Accepted 15 July 1982

ABSTRACT

The effect of chicken erythrocyte High Mobility Group protein 1 (HMG-1) on the enzymatic hydrolysis of purified double-stranded and single-stranded bacteriophage lambda DNA was studied. HMG-1 was found to inhibit the digestion of single- and double-stranded DNA by S1 nuclease and DNase I, respectively. HMG-1 increased the rate of hydrolysis of double-stranded DNA by micrococcal nuclease, particularly at low HMG-1/DNA ratios, and had little effect on the hydrolysis of single-stranded DNA by micrococcal nuclease, even at high HMG-1/DNA ratios. We also present a semi-quantitative estimate that HMG-1 and HMG-2 occur in chromatin from rapidly dividing, cultured rat hepatoma cells at about 8 times the level that they occur in adult rat liver chromatin.

INTRODUCTION

The High Mobility Group (HMG) proteins are a small group of nonhistone chromatin proteins (1) that are under intensive investigation in many laboratories. The interest in this laboratory in several of the HMG proteins stems from our finding that the high molecular weight HMG proteins (HMG-1, HMG-2, and HMG-E) are the most abundant nonhistone proteins from chromatins from several sources that are selectively retained by single-stranded DNA columns at 0.2 M NaCl (2,3). That suggests that HMG-1, HMG-2, and HMG-E possess preferential affinity for single-stranded DNA.

In the work described in this paper, we have examined the effect of chicken erythrocyte HMG-1 on the hydrolysis of purified bacteriophage lambda DNA by three enzymes: S1 nuclease, DNase I, and micrococcal nuclease. These studies were undertaken to compare the effects of HMG-1 with effects that other investigators have observed for thoroughly studied prokaryotic single-stranded DNA binding proteins and as a starting point in assessing the possible effects of HMG-1 on the digestion of DNA in chromatin. We also put our observation of enrichment of HMG-1 and HMG-2 in cultured hepatoma cells (2) on a semi-quantitative basis.

MATERIALS AND METHODS

Comparison of HMG-1 and HMG-2 levels in cultured hepatoma cells and adult rat liver. HTC cells, grown in suspension culture as described previously (5), were harvested by centrifugation and washed with TKM buffer (50 mM Tris-Cl, pH 7.5/25 mM KCl/5 mM MgCl₂) containing 0.25 M sucrose. Cells were broken by Potter-Elvehjem homogenization in TKM. Crude nuclei were pelleted by centrifugation at 5,000 g for 10 min; homogenized in TKM containing 0.25 M sucrose and 1% Triton X-100; centrifuged; homogenized in TKM containing 0.25 M sucrose; centrifuged; and stored at -20°. Nuclei were isolated from adult rat liver (Pel Freeze) with slight modifications of the method of Blobel and Potter (6). Nuclei recovered in the pellet from the Blobel and Potter centrifugation were washed with TKM containing 0.25 M sucrose; centrifuged at 5,000 g for 10 min; and homogenized in TKM containing 0.25 M sucrose and 1% Triton X-100. The nuclei were centrifuged; washed with TKM containing 0.25 M sucrose; centrifuged and stored at -20°. Chromatin was prepared from frozen rat liver or HTC cell nuclei by homogenizing thawed nuclei in 10 mM Tris-Cl (pH 7.5) three times and centrifuging at 5,000 g for 10 min to recover the chromatin after each homogenization.

Chromatin preparations from the two sources were adjusted to the same concentration (A_{260}). Solid NaCl was added to bring each to 0.35 M NaCl. Extracted proteins were obtained as a supernatant after centrifugation at 25,000 g for 30 min. Equal volumes of extracts from the two sources were then adjusted to 2% TCA. After 1 hr on ice, the samples were centrifuged for 10 min at 5,000 g to pellet the so-called LMG proteins (1). The supernatants were adjusted to 20% TCA and centrifuged after 1 hr on ice. The pellets (which contained HMG-1 and HMG-2) were washed twice with acetone and dissolved in equal volumes of electrophoresis buffer. Equal volumes were applied to 15% polyacrylamide/SDS slab gels. The gels were stained overnight in 0.25% Coomassie Blue/50% ethanol/10% acetic acid, destained in 25% ethanol/10% acetic acid, and scanned on a spectrodensitometer of the Schoeffel Instrument Corporation.

Preparation of [³H]-lambda DNA. *E. coli* K12, carrying thermo-inducible lambda-phage lysogen (λ CI 875^{ind-}), was used to obtain radioactively labeled λ -DNA. Cells were grown at 33° in K medium. After a culture achieved an optical density at 590 nm of 0.8, the temperature was raised to 50° for the induction of λ -phage, and 1 mCi of [methyl-³H]-thymidine (ICN Pharmaceuticals Inc.) was added per liter. After 30 min, the temperature was lowered to 37°; cells started lysing 60-75 min later. After complete lysis, 1 ml of chloroform was added per liter and the lysate was allowed to stand for 30 min at 4°. The

lysate was then subjected to centrifugation for 30 min at 13,000 g. The supernatant was recovered and centrifuged at 13,000 g for 12 hr. To each pellet was added 2 ml of 10 mM Tris-Cl (pH 7.5)/1 mM MgCl₂/0.1 M NaCl. After standing overnight at 4°, the suspended pellet was applied to a CsCl step gradient constructed with solutions of CsCl (in 10 mM Tris-Cl, pH 7.5/1 mM MgCl₂/0.1 M NaCl) of the following densities: 1.7, 1.55, 1.4, and 1.3. After centrifugation for 3 hr at 120,000 g, phage were collected from the interface between the solutions of densities 1.4 and 1.55. The phage were dialyzed against 10 mM Tris-Cl (pH 7.5)/1 mM EDTA/0.1 M NaCl, adjusted to 10 mM EDTA and 0.5% SDS, and incubated with proteinase K (10 µg/ml) for 3 hr at 37°. DNA was purified by chloroform:isoamyl alcohol (24:1) extraction and ethanol precipitation. It was stored as a stock solution in 1 mM Tris-Cl/1 mM EDTA (pH 7). Single-stranded DNA was prepared from double-stranded DNA by alkali denaturation as described by Studier (7). After neutralization with HCl, samples were clarified by centrifugation for 2 min at 15,000 g.

DNA concentrations were determined spectrophotometrically using $A_{260}^{0.1\%}$ of 21 and 30 for double-stranded and single-stranded DNA's, respectively. The DNA used in the work reported in this paper had a specific activity of 23,000 cpm/µg.

Preparation of [³H]-M13 DNA. E. coli K12 JM103, and M13 mp 7 phage were obtained from BRL and grown according to instructions provided by BRL. Radioactively labeled M13 DNA was obtained by infection of E. coli cultures that contained [³H]-thymidine. Culture supernatants were obtained by centrifugation for 30 min at 13,000 g. Phage were precipitated by bringing the supernatants to 4% polyethylene glycol-6000 and 0.5 M NaCl. The phage-containing pellet was obtained by centrifugation at 13,000 g for 30 min and subjected to equilibrium centrifugation in a CsCl gradient. The phage band from that centrifugation was collected and dialyzed against 20 mM Tris-Cl (pH 7.5)/0.1 mM EDTA/10 mM NaCl. SDS was added to a concentration of 0.1% and, after 30 min at 37°, DNA was purified by chloroform:isoamyl alcohol extraction, and ethanol precipitation. The purified DNA had a specific radioactivity of 12,000 cpm/µg.

Nucleases. S1 nuclease from Aspergillus oryzae was obtained from Calbiochem. DNase I and micrococcal nuclease were obtained from Worthington.

The following stock solutions were used. S1 nuclease: as supplied by Calbiochem, 3.4 mg (24 IU)/ml in 0.01 M sodium phosphate (pH 6.5)/50% glycerol. DNase I: 0.5 mg (1050 units)/ml in 1 mM HEPES (pH 7.0)/50% glycerol, as recommended by Hell et al. (8). Micrococcal nuclease: 200 µg (3050 units)/ml in 10 mM Tris-Cl, pH 7.5.

Preparation of HMG-1. Chicken erythrocyte HMG-1 was purified by phosphocellulose chromatography as described by Isackson et al. (9) and subsequently concentrated on phosphocellulose columns as described in the same paper. HMG-1 preparations were dialyzed against water in Spectropore tubing (Spectrum Medical Industries) with a molecular weight cutoff of 6-8,000. HMG-1 concentrations were determined spectrophotometrically, using $A_{280}^{0.1\%} = 0.76$ (M. D. Land and G. R. Reeck, unpublished result). The stock HMG-1 solutions were 300-700 $\mu\text{g/ml}$.

DNA digestions. Digestions of single-stranded λ -DNA or M13 DNA by S1 nuclease were carried out in 50 mM sodium acetate (pH 4.6)/1 mM ZnSO_4 /5% glycerol and either 10 or 100 mM NaCl. Reaction mixtures contained 3 μg DNA and from 0.001-0.006 units of enzyme. In the conditions we have used, S1 nuclease has been reported to selectively hydrolyze single-stranded DNA (10,11). We verified that for the commercial preparation of enzyme that we used in this work. Under reaction conditions that resulted in the hydrolysis to TCA solubility of over 1 μg of naked single-stranded λ -DNA (27,000 cpm), we detected no digestion of naked double-stranded DNA by the same enzyme preparation. That indicated not only that the enzyme preparation had excellent specificity but that our double-stranded DNA was essentially free of single-stranded regions.

Digestions of double-stranded DNA by DNase I were carried out in 10 mM HEPES (pH 7.5)/2.5 mM MgCl_2 and either 10 or 100 mM NaCl. At the lower ionic strength we used 0.05 μg of DNase I and at the higher ionic strength we used 0.5 μg of DNase I in each reaction. All reaction mixtures contained 2.4 μg DNA.

Digestions of single-stranded or double-stranded DNA by micrococcal nuclease were carried out in 50 mM HEPES (pH 7.5)/5 mM CaCl_2 and 10 or 100 mM NaCl. For studies with double-stranded DNA, the reaction mixtures contained 1.2-4.8 μg of DNA and from 5-80 ng of enzyme. For single-stranded DNA digestions, the reaction mixtures contained 2.4 μg DNA and 0.8 ng of enzyme.

Reaction mixtures had a total volume of 1.0 ml. All components other than enzyme were combined and incubated for 2 min at 37°. Enzyme was added to start the reactions, which were all carried out at 37°. At indicated times, 200 μl aliquots were removed and added to 200 μl of 10 mM EDTA (pH 7) to stop the reactions. Bovine serum albumin (Sigma Grade VII) was added at 0.2 mg/ml to act as a co-precipitant. The samples were placed on ice, and to each was added 400 μl of 40% trichloroacetic acid. After 10 min, the samples were centrifuged for 4 min at 15,600 g in an Eppendorf Microfuge. A portion (500 μl) of each supernatant was placed in 10 ml of Aquasol-2 (New England Nuclear) for liquid

scintillation counting in a Beckman LS-8000. In mock digestions performed in the absence of enzyme, 150-300 cpm/ml was observed in the supernatants, regardless of whether HMG-1 had been included. Thus, HMG-1 was free of detectable nuclease activity.

Assessment of possible interactions between HMG-1 and nucleases. A direct, physical approach -- gel permeation chromatography -- was used to examine whether HMG-1 binds directly to any of the three enzymes used in this work under the conditions in which digestions were performed. Experiments were conducted at a flow rate of 0.5 ml/min with a Beckman Model 332 HPLC system, a Model 160 absorbance detector (on a full scale absorbance setting of 0.02), a 7.5 x 100 mm TSK 2000 SW gel permeation column (Beckman Instruments). Molecules excluded from the beads in the column had a retention time of 2.5 min and molecules that had complete access to the interior of the beads had a retention time of 5.4 min.

We will describe in detail the experiment with micrococcal nuclease. The gel permeation column was equilibrated to 50 mM HEPES (pH 7.5)/5 mM $MgCl_2$ /0.1 M NaCl or to the same solvent containing micrococcal nuclease at a concentration of 10 μ g/ml. In the latter case, we verified that the amount of nuclease activity in the effluent stream was the same as the level of activity in the solvent reservoir. HMG-1 samples at 25 μ g/ml were prepared at the two solvent conditions (one including the enzyme and one lacking it). The samples that contained HMG-1 and micrococcal nuclease were incubated for a minimum of 15 min before injection. HMG-1 samples were injected in 50 μ l portions and the retention times of HMG-1 determined in triplicate. HMG-1 was diluted about 10-fold during the chromatography. The following retention times were obtained. HMG-1 with micrococcal nuclease in the solvent: 4.22 to 4.25 min. HMG-1 in the same solvent lacking micrococcal nuclease; 4.20 to 4.23 min. The formation of a complex between HMG-1 and the enzyme would lead to a lower retention time than that observed for HMG-1 in the absence of enzyme. Based on chromatography of proteins of known molecular weights on a TSK 2000 SW column (Dr. Philip Nordin, personal communication), a complex larger than HMG-1 by 17,000 (the molecular weight of micrococcal nuclease) would be expected to have a retention time of 3.8 min. Note that a complex need not be stable to be detected, since the presence of enzyme in the eluant would allow continual reassociation during chromatography. The fact that the retention time observed for HMG-1 in the presence of micrococcal nuclease is not less than the retention time for HMG-1 in the absence of micrococcal nuclease means that there is no interaction detected between the two proteins under these conditions. The

same result of no detectable interaction was obtained in analogously designed experiments in which S1 nuclease or DNase I was included in the solvent.

The ability of this gel permeation HPLC approach to demonstrate protein/protein interaction can be seen from the following. The retention time of Staphylococcal aureus Protein A (Pharmacia) in the absence of rabbit immunoglobulin G in the solvent was 4.3 min. The retention time of Protein A in the presence of immunoglobulin G in the solvent was found to be 2.4 min. (This experiment was conducted on a TSK 2000 SW column with slightly different V_0 value.)

RESULTS

Relative HMG-1 and HMG-2 contents in HTC cells and adult rat liver chromatin. We have previously reported (2) that the amounts of HMG-1 and HMG-2 in chromatin from cultured rat hepatoma cells (HTC cells) are substantially greater than the amounts of those proteins in adult rat liver chromatin, but we did not estimate in quantitative terms the relative contents in chromatin from the two sources. The measurements described here are designed to provide a semi-quantitative estimate. In the isolations from HTC cells and from adult rat liver, the nuclei from the two sources were exposed to similar conditions. Chromatins from the two sources were then extracted with 0.35 M NaCl under identical conditions, and a fraction enriched in HMG-1 and HMG-2 was obtained by TCA precipitations. Samples derived from equivalent amounts of chromatin were electrophoresed, and the stained gel was scanned with a densitometer. Scans of the regions that contained HMG-1 and HMG-2 (Figure 1) indicated that the content of HMG-1 and HMG-2 in HTC cell chromatin is 8.5 times greater than the content of those proteins in adult rat liver chromatin.

Influence of HMG-1 on the digestion of single-stranded DNA by S1 nuclease. Figure 2 shows time course digestions of single-stranded bacteriophage λ -DNA in 50 mM sodium acetate (pH 4.5)/1 mM ZnSO_4 /5% glycerol/0.1 M NaCl. The digestions proceed more slowly in the presence of HMG-1 than in its absence. We calculated the rates of digestion at different HMG-1 levels by linear regression of the data in each time course through 20 min. Figure 3 shows the dependence of the rate of digestion upon the level of HMG-1 in the reaction mixtures. Essentially complete inhibition was achieved at a weight ratio of 4:1 (HMG-1: DNA). A comparable result was obtained when single-stranded M13 DNA was digested by S1 nuclease in the presence of various amounts of HMG-1 (not shown). Comparable patterns of inhibition of S1 nuclease digestion of

Figure 1. Densitometric scans of SDS/polyacrylamide gel electrophoretograms. The samples were derived from identical amounts of chromatin from HTC cells and rat liver. The densitometric scans of the 2 samples were obtained under identical conditions. Relative areas were determined by tracing the patterns onto paper, cutting out each HMG-1/HMG-2 peak, and weighing.

Figure 2. Hydrolysis of single-stranded λ -DNA by S1 nuclease in the presence of several concentrations of HMG-1. The amounts of HMG-1 (in $\mu\text{g/ml}$) were: 0 ($\circ$); 2 (Δ); 4 ($\square$); 6 ($\bullet$); 8 ($\blacktriangle$); and 10 ($\blacksquare$). The reaction mixtures contained 3 μg of DNA and 0.006 units of enzyme. The solvent was 50 mM sodium acetate (pH 4.6)/1 mM ZnSO_4 /5% glycerol/100 mM NaCl. Best fitting straight lines passing through the origin were determined by least-squares calculations.

Figure 3. Inhibition by HMG-1 of the hydrolysis of single-stranded λ -DNA by S1 nuclease. The reaction mixtures contained 3 μ g of DNA and 0.006 units of enzyme. The solvent was 50 mM sodium acetate (pH 4.6)/1 mM ZnSO_4 /5% glycerol/100 mM NaCl. The control rate was 890 cpm released/min.

single-stranded λ -DNA were observed at a lower enzyme level (0.001 unit per reaction) and at lower ionic strength (0.01 M NaCl in the reaction mixture) (not shown).

Influence of HMG-1 on the digestion of double-stranded DNA by DNase I.

The enzymatic properties of pancreatic DNase I are strongly influenced, both quantitatively and qualitatively, by the type and concentration of divalent cation (12). Under the reaction conditions we have used, the enzyme strongly preferred double-stranded DNA, and we have therefore limited our studies to that form of DNA. The two reaction conditions we used differ only in NaCl concentration. At the lower NaCl concentration (10 mM) the reaction conditions resemble those used in digestion of chromatin by DNase I (reference 13). We have also used 0.1 M NaCl, in which we would anticipate a weaker interaction of HMG-1 with double-stranded DNA (3).

Time course digestions were carried out and rates of digestion were calculated by linear regression of the data in the linear portions of the reactions (through 15 min). The dependence of the rates of digestion (in both 10 and 100 mM NaCl) upon the level of HMG-1 in the reactions is shown in Figure 4.

Influence of HMG-1 on the digestion of single-stranded and double-stranded DNA's by micrococcal nuclease. This enzyme readily digests double-stranded DNA but actually hydrolyzes single-stranded DNA more rapidly (14). We have examined the influence of HMG-1 on the digestion of each type of DNA. The reactions were carried out in 50 mM HEPES (pH 7.5)/5 mM CaCl_2 containing either 10 or 100 mM NaCl. Again we show the results of linear regression analysis of

Figure 4. Inhibition of the hydrolysis of double-stranded λ -DNA by DNase I. The reaction mixtures contained 2.4 μ g of DNA. The solvent was 10 mM HEPES (pH 7.5)/2.5 mM MgCl_2 , and either 10 mM NaCl (closed circles) or 100 mM NaCl (open circles). At the lower NaCl concentration, we used 0.05 μ g of enzyme and the control rate was 560 cpm released/min. At the higher NaCl concentration, we used 0.5 μ g of enzyme and the control rate was 660 cpm released/min.

time course digestions.

Figure 5 shows that HMG-1 does not inhibit the hydrolysis of single-stranded DNA by micrococcal nuclease. If anything, there is a slight stimulation of the rate of digestion in the presence of HMG-1. Figure 6 shows the effect of HMG-1 on the digestion of double-stranded DNA by the enzyme. Here the effect is clearly stimulatory, with the greatest stimulation occurring at around 10 μ g/ml. Under all conditions that we have examined (including 10 mM rather than 100 mM NaCl), the rate of digestion of double-stranded DNA was increased by the presence of HMG-1. The stimulatory effect is less pronounced at high levels of HMG-1 (see Figure 6).

Figure 5. Influence of HMG-1 on the hydrolysis of single-stranded λ -DNA by micrococcal nuclease. The reaction mixtures contained 2.4 μ g of DNA and 0.8 ng of enzyme. The solvent was 50 mM HEPES (pH 7.5)/5 mM CaCl_2 /100 mM NaCl. The control rate was 720 cpm released/min.

Figure 6. Influence of HMG-1 on the hydrolysis of double-stranded λ -DNA by micrococcal nuclease. The reaction mixtures contained 2.4 μ g of DNA and 20 ng of enzyme. The solvent was 50 mM HEPES (pH 7.5)/5 mM CaCl_2 /100 mM NaCl. The control rate was 360 cpm released/min.

DISCUSSION

Levels of HMG-1 and HMG-2 in HTC cell and rat liver chromatin. Data presented in Figure 1 indicate that the amounts of HMG-1 and HMG-2 in HTC cell chromatin are roughly an order of magnitude greater than the amounts of those two proteins in chromatin from adult rat liver. There could be a variety of explanations for that. The one we favor is that the enrichment in HTC cell chromatin is due to the fact that the HTC cells were rapidly proliferating when harvested, whereas the cells in adult rat liver are essentially not proliferating. It should be noted that Gordon et al. (15) have demonstrated with chick embryo fibroblasts that a substantial portion of HMG-1 and HMG-2 is not stably associated with nuclei. The procedures with which we have isolated nuclei presumably lead to recovery of only the stably bound HMG-1 and HMG-2.

Seyedin and Kistler (16) have reported that the amount of HMG-2 (but not of HMG-1) is correlated with the level of cell proliferation in several rat organs. The discrepancy between their results and our finding of comparable enrichment of both HMG-1 and HMG-2 in rapidly proliferating rat cells may simply be due to the substantial differences in experimental protocols. Seyedin and Kistler extracted HMG-1 and HMG-2 from homogenates of entire organs with H_2SO_4 , whereas we extracted HMG-1 and HMG-2 from isolated chromatin with NaCl. HMG-1 and/or HMG-2 appear to occur in the cytoplasm (as well as nuclei) of intact cells (17), and both proteins can be isolated from the cytosolic fraction of broken cells (18). Differences in the HMG-1/HMG-2 ratios in nuclei and cytosol or differences in extraction efficiencies could possibly

explain the discrepancy between the observations of Seyedin and Kistler and data in Figure 1.

We have not attempted to determine the absolute amounts of HMG-1 or HMG-2 in HTC cell or rat liver chromatin. An earlier calculation of the amount of HMG-1 in HTC cell chromatin (2) was based on a stated assumption of 100% yield in a several-step isolation scheme. We now believe that our yield was much lower than 100%. In that isolation scheme (2), we used regular pore dialysis tubing that had been very extensively boiled before use to remove impurities. We now know that substantial amounts of HMG-1 are lost from such tubing during dialysis, and we therefore believe that the HMG-1 content in HTC cell chromatin is probably over 10^6 molecules per haploid genome (instead of the 5×10^5 we estimated earlier).

HMG-1 influence on DNA digestions. The effects of HMG-1 that we have observed on the digestion of purified DNA by S1 nuclease, DNase I, and micrococcal nuclease could, in principle, be due to interaction of HMG-1 with DNA, with the enzymes, or both. HMG-1 is known to bind to DNA (3,19,20) but does not appear to interact with any of the three enzymes under the solvent conditions we have used (see Materials and Methods). Thus we attribute changes in rates of DNA hydrolysis to binding of HMG-1 to DNA. For the inhibition of hydrolysis of single-stranded DNA and double-stranded DNA by S1 nuclease and DNase I, respectively, it is plausible that by binding to DNA, HMG-1 simply hinders access of the enzymes to their substrate and thus decreases the rates of DNA digestion. The fact that HMG-1 inhibits the S1 nuclease digestion of M13 DNA (which occurs within virions as single-stranded molecules) rules out the possibility that the effect of HMG-1 on the S1 nuclease digestion of single-stranded λ -DNA could be due to its promoting re-annealing into S1 nuclease-resistant double-stranded molecules.

In its inhibition of the digestion of single-stranded DNA by S1 nuclease, HMG-1 resembles two prokaryotic single-stranded DNA binding proteins. The gene 32 protein of bacteriophage T4, the most thoroughly studied of such proteins, has been shown to inhibit the hydrolysis of single-stranded DNA by an *E. coli* endonuclease (21), by the exonuclease activity of T4 DNA polymerase (22), by a *Neurospora crassa* endonuclease (23), and by S1 nuclease (23). The *E. coli* single-stranded DNA binding protein has also been shown to inhibit the hydrolysis of single-stranded DNA by S1 nuclease (24). Based in part on such results, Gold and his colleagues (25) have suggested that the gene 32 protein serves to protect single-stranded DNA regions against attack by nucleases. A similar proposal for the high molecular weight HMG proteins would seem to merit

consideration. In that context, the enrichment of HMG-1 and HMG-2 in rapidly proliferating HTC cells is of interest.

The effects of HMG-1 on the hydrolysis of DNA by micrococcal nuclease are difficult to rationalize. Even at HMG-1 levels where we would expect a high density of HMG-1 molecules on single-stranded DNA, no inhibition was observed (Figure 5). It is possible that the sites on single-stranded DNA to which HMG-1 and micrococcal nuclease bind do not overlap, or, perhaps more likely, that micrococcal nuclease binds much more tightly to DNA than does HMG-1 and therefore readily displaces HMG-1.

The enhanced rate of digestion of double-stranded DNA in the presence of HMG-1 is intriguing. Given the preference of micrococcal nuclease for single-stranded DNA (14), the preferential affinity of HMG-1 for single-stranded DNA at 0.1 M NaCl (3,20), and the lack of inhibition of the hydrolysis of single-stranded DNA by the enzyme in the presence of HMG-1 (Figure 5), it is possible that the stimulation of hydrolysis of double-stranded DNA by micrococcal nuclease in the presence of HMG-1 is due to HMG-1's exposing regions of single-stranded DNA to the enzyme. We see two problems with such a rationalization. First, it would be hard to explain the decreased stimulation that we observe as HMG-1/DNA ratios are increased (see Figure 6). Second, proposing such a mechanism of stimulation would be essentially equivalent to requiring that HMG-1 act as a melting protein — that is, be capable of lowering the melting temperature of double-stranded DNA's. Jahaverian et al. (26) have in fact reported that calf thymus HMG-1 is capable of lowering the melting temperatures of poly dA-dT and of T7 DNA under certain conditions. In extensive studies of the thermal denaturation of poly dA-dT in the presence of chicken erythrocyte HMG-1, we have been unable to demonstrate a depression of melting temperature (27; Isackson and Reeck, manuscript in preparation). We consider it unlikely, therefore, that the effect of chicken erythrocyte HMG-1 on the hydrolysis of double-stranded DNA by micrococcal nuclease is due to HMG-1's acting as a melting protein. As an alternate explanation, we propose that HMG-1 places DNA in a conformation that is particularly favorable for the binding of the enzyme to the DNA. High HMG-1 levels (above 10 $\mu\text{g/ml}$ in Figure 6) might change the DNA conformation further, to one that is somewhat less suitable for binding of the enzyme.

Levy et al. (28) have shown that nucleosomes enriched in the trout testis high molecular weight HMG protein (HMG-T) are preferentially released from chromatin by digestion with micrococcal nuclease. Jackson et al. (29) have obtained an analogous result with mouse myeloma chromatin: in the early stages

of digestion with micrococcal nuclease, nucleosomes are released that are enriched in HMG-1 and HMG-2. It is possible that the stimulation by HMG-1 of the digestion of naked double-stranded DNA by micrococcal nuclease observed in our work is related to the preferential attack by that enzyme on nucleosomes that contain high molecular weight HMG proteins.

ACKNOWLEDGMENTS

We thank Professor Vernon Bode (Division of Biology) for advice on purification of bacteriophage λ , Professor Thomas Currier (Department of Plant Pathology) for similar advice on M13, and Professor Lynn Bates (Department of Grain Science) for use of the densitometer. This work was supported by the Kansas Agricultural Experiment Station and grants from the National Institutes of Health (R01-CA-17782, R01-GM-29203, and K04-CA-00425). This is contribution number 81-430-j, Kansas Agricultural Experiment Station.

*To whom correspondence should be addressed.

REFERENCES

1. Goodwin, G. H. and Johns, E. W. (1973) *Eur. J. Biochem.* 40, 215-219
2. Bidney, D. L. and Reeck, G. R. (1978) *Biochem. Biophys. Res. Commun.* 85, 1211-1218
3. Isackson, P. J., Fishback, J. L., Bidney, D. L., and Reeck, G. R. (1979) *J. Biol. Chem.* 254, 5569-5572
4. Alberts, B. M. and Frey, L. (1970) *Nature* 227, 1313-1318
5. Bidney, D. L. and Reeck, G. R. (1978) *Biochim. Biophys. Acta* 521, 753-761
6. Blobel, G. and Potter, V. R. (1966) *Science* 154, 1662-1665
7. Studier, F. W. (1965) *J. Mol. Biol.* 11, 373-390
8. Hell, A., Birnie, G. D., Slimming, T. K., and Paul, J. (1972) *Anal. Biochem.* 48, 369-377
9. Isackson, P. J., Debold, W. A., and Reeck, G. R. (1980) *FEBS Letters* 119, 337-342.
10. Ando, T. (1966) *Biochim. Biophys. Acta* 114, 158-168
11. Vogt, V. M. (1973) *Eur. J. Biochem.* 33, 192-200
12. Laskowski, M., Sr. (1971) in *The Enzymes*, P. Boyer, ed., Vol. IV, pp. 289-311 Academic Press, New York
13. Weintraub, H. and Groudine, M. (1976) *Science* 193, 848-856
14. Dirksen, M. L. and Dekker, C. A. (1960) *Biochem. Biophys. Res. Commun.* 2, 147-152
15. Gordon, J. S., Bruno, J., and Lucas, J. J. (1981) *J. Cell Biol.* 88, 373-379
16. Seyedin, S. M. and Kistler, W. S. (1979) *J. Biol. Chem.* 254, 11264-11271
17. Bustin, M. and Neihart, N. K. (1979) *Cell* 16, 181-189
18. Isackson, P. J., Bidney, D. L., Reeck, G. R., Neihart, N. K., and Bustin, M. (1980) *Biochemistry* 19, 4466-4471
19. Shooter, K. V., Goodwin, G. H., and Johns, E. W. (1974) *Eur. J. Biochem.* 47, 263-270

20. Isackson, P. J., Clow, L. C., and Reeck, G. R. (1981) FEBS Letters 125, 30-34
21. Wu, J. R. and Yeh, Y. C. (1973) J. Virol. 12, 758-765
22. Huang, W. M. and Lehman, I. R. (1972) J. Biol. Chem. 247, 3139-3146
23. Gold, L., Lemaire, G., Martin, C., Morrissett, H., O'Conner, P., O'Farrell, P., Russel, M., and Shapiro, R. (1977) in Nucleic Acid-Protein Recognition, H. J. Vogel, ed., pp. 91-112 Academic Press, New York
24. Christiansen, C. and Baldwin, R. L. (1977) J. Mol. Biol. 115, 441-454
25. Gold, L., O'Farrell, P. Z., and Russel, M. (1976) J. Biol. Chem. 251, 7251-7262
26. Javaherian, K., Sadeghi, M., and Liu, L. F. (1979) Nucleic Acids Res. 6, 3569-3580
27. Isackson, P. J. (1980) Ph.D. Dissertation, Kansas State University
28. Levy, B. W., Connor, W., and Dixon, G. H. (1979) J. Biol. Chem. 254, 609-620
29. Jackson, J. B., Pollock, J. M., Jr., and Rill, R. L. (1979) Biochemistry 18, 3739-3748