for example, highly repetitive sequences are relatively enriched in m^{5}dC content (Solage & Cedar, 1978; Kastan et al., 1982; Ehrlich & Wang, 1981). Therefore, it is reasonable that the preferential association of m^{5}dC with nuclease-resistant regions of DNA could result from an intrinsic SN resistance of a few repetitive sequences in which a significant proportion of methylation occurs.

On a more general level, because most (perhaps greater than 90%) of m^{5}dC occurs in the sequence m^{5}dC-dG (Ehrlich & Wang, 1981), the proportion of m^{5}dC residues appearing in a dC·dG-rich region is greater than the proportion of unmethylated dC residues appearing in that region. Since dC·dG-rich regions are relatively more resistant to SN digestion, this higher proportion of m^{5}dC residues occurring in dC·dG-rich regions could explain the even higher nuclease resistance of m^{5}dC·dG-rich regions.

Registry No. SN, 9013-53-0; m^{5}dC, 838-07-3.

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Hierarchy of Binding Sites for Chromosomal Proteins HMG 1 and 2 in Supercoiled Deoxyribonucleic Acid

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ABSTRACT: The interaction of chromosomal proteins HMG 1 and 2 with various DNA structures has been examined with plasmid pPst-0.9, which contains DNA sequences that can form the Z-DNA conformation and palindromic sequences that can form cruciform structures. Direct binding and competition experiments with ^{32}P-labeled plasmid indicated that proteins HMG 1 and 2 preferentially bind to supercoiled form I DNA as compared to double-stranded linear DNA. The preferential binding to form I is due to the presence of single-stranded regions in this DNA. The binding of HMG 1 and 2 to the form I plasmid results in inhibition of S_1 nuclease digestion in a selective manner. The B-Z junction is preferentially protected as compared to the cruciform, which in turn is more protected than other minor S_1-sensitive structures present in pPst-0.9. Our results indicate that the binding of HMG 1 and 2 proteins to DNA is not random in that HMG 1 and 2 can distinguish between various S_1 nuclease sensitive sites in the plasmid. The existence of a hierarchy of DNA binding sites for these proteins suggests that they can selectively affect the structure of distinct regions in the genome.

Structural alteration in DNA may play a key role in the regulation of gene expression. Distinct DNA conformations may be facilitated and stabilized by various cellular proteins. Chromosomal proteins HMG 1 and 2 are potential candidates for affecting structural featues of DNA. These proteins are relatively abundant in both the nucleus and cytoplasm of cells and are ubiquitously distributed among various eukaryotic kingdoms, and their primary and tertiary structures are relatively conserved during evolution [see Johns (1982) for review]. They can distinguish between single-stranded and double-stranded DNA (Isackson et al., 1979), unwind the DNA double helix (Javaherian et al., 1978, 1979; Yoshida & Shimura, 1984), and influence the enzymatic digestion of DNA by various nucleases (Shasti et al., 1982). In chromatin, they seem to be bound to the linker region between nucleosomes. In spite of these findings, the cellular role of HMG 1 and 2 is not clear. It has been reported that cell division brings about quantitative changes in HMG 1 and 2 (Seyadin & Kistler, 1979). Microinjection of ant-HMG 1 and anti-HMG 2 antibodies into the nucleus of amphibian oocytes inhibits the highly active transcription on the lampbrush chromosome loops and brings about a retraction of the loops into the chromomeres (Kleinschmidt et al., 1983). In nuclei of somatic cells, however, antibody microinjection did not produce a noticeable effect on RNA synthesis mediated by RNA polymerase II (Einck & Bustin, 1983).

Understanding the interactions between HMG 1 and 2 and various DNA structures may provide insights into the cellular role of these proteins. In this paper we address this question by studying the binding of HMG 1 and 2 to a plasmid that contains DNA sequences that can adopt various non-B structures including Z DNA and cruciform. Analysis of the HMG binding to the supercoiled and linearized plasmids in conjuction with various nuclease digestions leads us to conclude that HMG 1 and 2 can detect differences among similar DNA structures, implying a hierarchy of DNA binding sites for this protein.

Materials and Methods

Materials. Plasmid pPst-0.9 is a recombinant plasmid containing a part of human cardiac muscle actin gene that includes $(dT\text{-}dG)_{25}\cdot(dC\text{-}dA)_{25}$ (Hamada et al., 1982a). The plasmid was propagated in *Escherichia coli* C600. Form I plasmid DNA was purified by CsCl–ethidium bromide centrifugation. The plasmid was linearized by digestion with *Pvu*II. Single-stranded DNA was generated by rapid cooling of boiled *E. coli* DNA. Plasmid pBR322 was obtained from Bethesda Research Laboratories. HMG 1 and 2 proteins were isolated from calf thymus and purified as described previously (Romani et al., 1979). The purified proteins were radiolabeled with ^3H by reductive methylation (Moore & Crichton, 1973). The ^3H-labeled proteins reacted with antibodies elicited against the unlabeled proteins (Bustin et al., 1981). Histones were prepared from calf thymus by acid extraction as described previously (Bustin, 1973).

Filter Binding Assay. Plasmid pPst-0.9 labeled with [^{32}P]orthophosphate in vivo (sp act. $\sim 6 \times 10^4$ cpm/μg) was incubated with various amounts of HMG 1 and 2 in 100 μL of 30 mM sodium acetate (pH 4.6)–1 mM $ZnCl_2$ for 1 h at 4 °C. The mixture was then applied on a nitrocellulose filter (Schleicher and Schuell, BA85). The filter was washed with the same buffer 3 times and dried, and the radioactivity remaining on the filter was counted in aquasol.

S_1 Nuclease Protection Assay. Form I pPst-0.9 DNA (5 μg) was first incubted with various amounts of HMG 1 and 2 in 100 μL of 30 mM sodium acetate (pH 4.6)–1 mM $ZnCl_2$ at 4 °C for 1 h. A 200-μL aliquot of the same buffer and 5 units of S_1 nuclease (Bethesda Research Laboratory) were then added to the mixture. The mixture was incubated at 37 °C for 30 min. S_1 nuclease digestion was terminated by the addition of 20 mM ethylenediaminetetraacetic acid (EDTA). Aliquots taken from themixture before and after S_1 nuclease treatment were analyzed on 1% agarose gel. For mapping of the initial S_1 cut, the S_1-treated DNAs were extracted with phenol, dialyzed, precipitated with ethanol, and restricted with *Eco*RI. The *Eco*RI digests were fractionated on a 1% agarose gel.

Other Methods. Proteins were examined in 18% polyacrylamide gels, containing 0.1% sodium dodecyl sulfate (Laemmli, 1970). Densitometry of negatives taken from stained agarose gels was done with a Beckman DU-8 spectrophotometer.

Results

Plasmid pPst-0.9. Plasmid pPst-0.9 was constructed by inserting a 0.9-kb sequence derived from the human cardiac muscle actin gene at the *Pst*I sequence of plasmid pBR322. The gene insert contains a simple repetitive site $(dT\text{-}dG)_{25}$, which has a Z-DNA forming potential (Hamada et al., 1982a,b). Indeed, at low ionic strength (<30 mM NaCl) anti-Z-DNA antibodies bind specifically to form I pPst-0.9. The generation of the Z-DNA conformation is accompanied

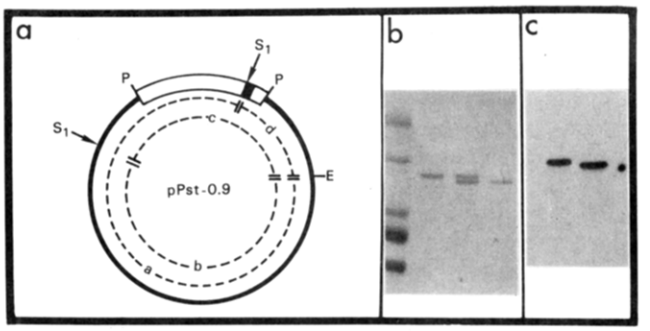

FIGURE 1: Plasmid pPst-0.9 and HMG 1 and 2 proteins. (a) Schematic representation of the pertinent features of pPst-0.9. P denotes the *Pst*I restriction sites at which the 0.9-kb human DNA fragment (open frame) was inserted. The black block in the insert indicates the location of $(dT\text{-}dG)_{25}\cdot(dC\text{-}dA)_{25}$. S_1 denotes the main cutting sites by S_1 nuclease within the insert and at position 3065 in the pBR322 section of the plasmid. E denotes the *Eco*RI site. (a and d) Fragments generated when initial S_1 attack is in the 0.9-kb insert; (b and c) fragments generated when initial S_1 cut is at nucleotide 3064. (b) Purity of HMG 1 and HMG 2 demonstrated by electrophoresis in 18% sodium dodecyl sulfate (SDS) gels. From left, molecular weight markers, HMG 1, HMG 1 and HMG 2, and HMG 2. (c) Autoradiogram of HMG 1 (left) and HMG 2 (right) labeled with ^3H by the sodium borohydrate procedure. Molecular weight markers were ovalbumin (43 000), α-chymotrpysin (25 700), β-lactoglobin (18 400), lysozyme (14 300), and cytochrome *c* (12 300).

by the appearance of an S_1 nuclease sensitive region that maps at multiple, closely spaced, sites within one of the two B–Z junctions (Hamada et al., 1984). Since plasmid pPst-0.9 is a pBR322 derivative, it contains a number of additional S_1 nuclease sensitive sites, the most prominent of which is the palindromic sequence at residue 3065 that forms a cruciform under negative supercoiling (Lilley, 1980). The site of S_1 digestion in form I pPst-0.9 can be identified by restricting the S_1 nuclease digested plasmid with *Eco*RI. As shown in the diagram presented in Figure 1, S_1 nuclease cleavage at the B–Z junction followed by *Eco*RI digestion will generate fragements a (4.3 kb) and d (0.87 kb) while, if the initial S_1 nuclease digestion occurred at the cruciform, the fragments generated will be b (3.1 kb) and c (2.1 kb). By virtue of its potential Z DNA forming sequences and distinguishable S_1 nuclease sensitive sites, this plasmid is a suitable substrate for investigations on the binding of proteins to various DNA conformations. The non-histone chromosomal proteins HMG 1 and HMG 2 isolated from calf thymus were pure as determined by electrophoresis in polyacrylamide gels (see Figure 1b) and by immunological methods (Bustin et al., 1970; Romani et al., 1980). As a first step, we determined whether HMG 1 and 2 can distinguish between different DNA conformations of plasmid pPst-0.9.

Preferential Binding of HMG to Supercoiled DNA. The binding of HMG 1 and 2 to ^{32}P-labeled supercoiled pPst-0.9 was compared to that of the same plasmid linearized by digestion with *Pvu*II. This enzyme was chosen because it does not generate single-stranded ends, which may effect the binding of the protein to the linearized plasmid as well as the binding of the DNA to the nitrocellulose membrane. The data presented in Figure 2 clearly indicate that the HMG proteins bind significantly better to supercoiled DNA than to the linearized DNA. It can be seen that under conditions where 50% of the supercoiled DNA is bound (10 ng of HMG 1 and 2 added) there is no detectable binding to linear DNA. It is necessary to add more than 50 ng of HMG 1 and 2 to get 50% binding of linearized DNA. At this protein to DNA input ratio the binding of superhelical DNA has reached saturation. The preferential binding to the supercoiled DNA is dependent on

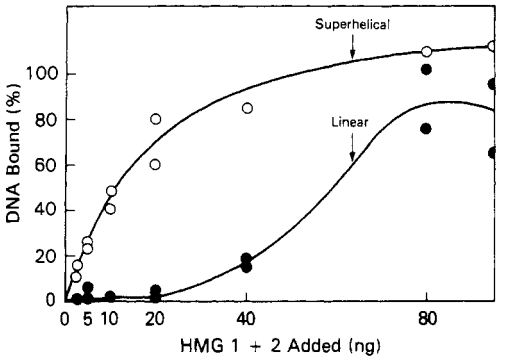

FIGURE 2: Preferential binding of superhelical DNA by HMG 1 and 2. Mixtures containing the indicated amounts of HMG 1 and 2 were incubated with either superhelical (O) or linear (●) ^{32}P-labeled pPst-0.9, and the amount of DNA bound by the protein was determined by filter binding (see Materials and Methods).

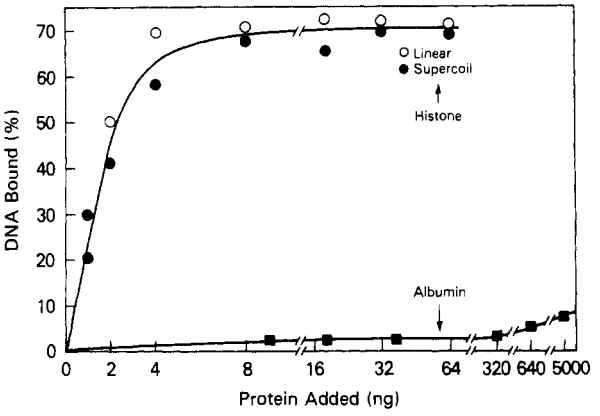

FIGURE 3: Binding of pPst-0.9 to non-HMG 1 and 2 proteins. Supercoiled (●, ■) or linear (O) ^{32}P-labeled plasmid was incubated with either histones (●, O) or albumin (■). Binding was determined by the filter method. Note that histones do not distinguish between linear and supercoiled DNA and that albumin did not bind to either form of DNA.

some specific interaction between the HMG proteins and the DNA since control experiments involving addition of histones to the two forms of DNA indicated that histones bind equally well to form I and form III (linear) DNA (see Figure 3). The data presented in Figure 3 also indicate that control proteins such as bovine serum albumin do not cause retention of the DNA on the filter even when added in large excess. We note however that when the albumin concentration reached 10 μg, over 60% of the DNA were retained on the filter.

The higher affinity of HMG 1 and 2 for superhelical as compared to linear DNA was observed also in experiments where the binding of ^{3}H-labeled HMG 1 and 2 (see Figure 1c) to unlabeled DNA was measured. In these experiments a mixture containing [^{3}H]HMG 1 and 2 and ether supercoiled or linear DNA was centrifuged at 100000*g* for 30 min in a Beckman airfuge. The nucleoprotein complexes sedimented while the free HMG protein remained in the supernatant. Using this experimental protocol, we were able to estimate that about 35% of the input-labeled [^{3}H]HMG 1 and 2 bound to DNA and that linearized DNA bound approximately 55% as much HMG as superhelical DNA.

The competition experiments presented in Figure 4 bring further evidence for the ability of the HMG to distinguish between supercoiled and linear DNA. In these experiments various preparations of unlabeled DNA were used to inhibit the binding of the HMG proteins to ^{32}P-labeled supercoiled DNA. It can be seen that 50% inhibition was obtained with 0.05 μg of form I of either plasmid pPst-I or of plasmid

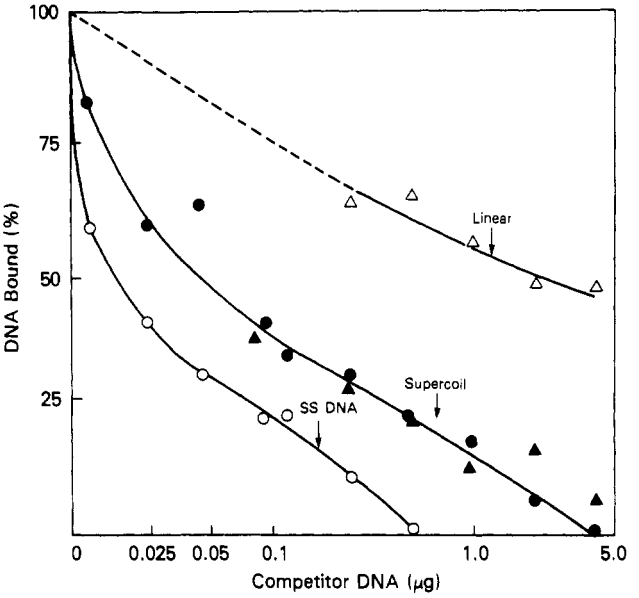

FIGURE 4: Inhibition of HMG binding to supercoiled ^{32}P-labeled pPst-0.9 by various types of DNA. The binding of 10 ng of HMG to 50 ng of ^{32}P-labeled pPst-0.9 was inhibited by various amounts of unlabeled DNA [(O) single-stranded *E. coli* DNA, (●) superhelical pPst-0.9, (▲) superhelical pBR322, (△) linear pPst-0.9].

pBR322. It was necessary to use 10 times more linear DNA to obtain the same degree of inhibition. The ability of plasmid pBR322 to compete for HMG binding indicates that the binding of the proteins to plasmid pPst-0.9 is not dependent on some unique features that are present exclusively in this plasmid. Indeed, it has been reported that form I pBR322 contains both S_1 nuclease sensitive sites and some Z-DNA characteristics (Nordheim et al., 1982). One obvious target for HMG binding in supercoiled DNA is regions that may adopt single-stranded characteristics, recognizable by S_1 nuclease. Such regions are most prominent at the B–Z junctions (Singleton et al., 1984) and in cruciform structures (Lilley, 1980; Panayotatos & Wells, 1981). These S_1-sensitive structures (as well as other minor S_1-sensitive structures in pBR322) are known to be dependent on supercoiling and are not present in linear, double-stranded DNA. Indeed, the data presented in Figure 4 indicate that single-stranded *E. coli* DNA was the most effective competitor for the HMG binding. Less than 0.02 μg of *E. coli* single-stranded DNA competed for HMG binding more effectively than 0.05 μg of form I plasmid pPst-0.9.

The data presented so far suggest therefore that the HMG proteins can distinguish between supercoiled and linear DNA because the torsional constrains present in form I DNA induce single-stranded properties to specific regions of the plasmid. If indeed this is the case, then it could be expected that the binding of HMG to these regions would be reflected in their susceptibility to S_1 nuclease digestion.

Selective Protection of S_1 Nuclease Sensitive Sites. The data presented in Figure 5 indicate that chromosomal proteins HMG 1 and 2 protect plasmid pPst-0.9 from S_1 digestion. In the figure, lanes 1–3 are control experiments containing mixtures of plasmid and HMG proteins. The plasmid preparation contained a mixture of supercoiled monomers and dimers. Addition of HMG did not result in any DNA cleavage; however, at high protein to DNA ratios (64 or 128 HMGs/DNA), the mobility of the supercoiled DNA decreased (lanes 2 and 3). Digestion of pPst-0.9 with S_1 nuclease (lanes 6–14) results in nicking and subsequent linearization of both the monomer and dimer plasmids. The S_1 digestion appeared

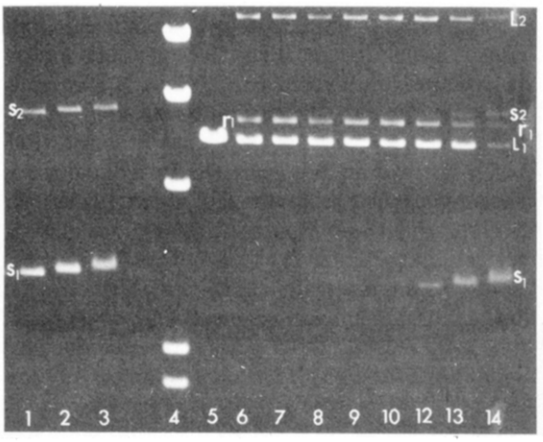

FIGURE 5: HMG protects pPst-0.9 from S_1 digestion. Plasmid pPst-0.9 was incubated with various amounts of HMG and digested with S_1 as described under Materials and Methods: (lanes 1–3) no S_1 addition (HMG to DNA ratio of 32, 64, and 128, respectively); (lane 4) molecular weight markers (λDNA × *Hin*dIII; from top, 9.4, 6.6, 4.4, 2.3, and 2.0 kb); (lane 5) form III (marked as L_1) pPst-0.9; (lanes 6–14) pPst-0.9 digested with S_1. The molar ratio of HMG to DNA in lanes 6–10 and 12–14 was 0, 2, 4, 8, 16, 32, 64, and 128, respectively. DNAs in lanes 1–3 and 6–14 were not extracted with phenol prior to electrophoresis. S_1 and S_2, supercoiled monomer and dimer; L_1 and L_2, linear monomer and dimer; r_1, relaxed monomer. Lane 11 is missing due to mislabeling in the figure.

to be very specific since the linear form was the end product of the digestion.

Chromosomal proteins HMG 1 and 2 protect plasmid pPst-0.9 from S_1 nuclease digestion as evidenced by the presence of form I monomer and dimer after the nuclease treatment (lanes 10–14). The degree of protection could be correlated with the amount of protein added to DNA. The decrease in mobility was observed only with form I DNA and not with the relaxed or linear DNA (lanes 2, 3, 13, and 14). This finding supports our previous observation, obtained by the filter assay, regarding preferential binding of HMG 1 and 2 to form I DNA. The ability of HMG 1 and 2 to protect various S_1 nuclease sensitive sites in pPst-0.9 (B–Z junction, palindrome at residue 3065, and a number of minor S_1 sites) can be determined by restricting the nuclease-treated plasmid with *Eco*RI. For example, the initial cleavage by S_1 nuclease at the two major S_1 nuclease sensitive sites results in the appearance of bands identified as a–d in both Figure 1 and Figure 6. Protection of a particular site would lower the relative amount of a pair of bands. The S_1 digests shown in Figure 5 were further digested with *Eco*RI and analyzed on an agarose gel (inset in Figure 6). The gel was scanned to quantitate the amount of each S_1–*Eco*RI subfragment (Figure 6), and the relative protection from S_1 nuclease confered by HMG 1 and 2 to various S_1 sensitive sites is summarized in Figure 7. The results indicated that some S_1 sites are preferentially protected compared to other S_1 sites. This is most strikingly demonstrated when the protection of the B–Z junction is compared to that of total S_1 sites; the whole plasmid is not significantly protected from S_1 digestion at a molar input of HMG 1 and 2 to DNA of 16 (Figure 5, lane 10, and Figure 7), while the B–Z junction is almost completely protected at this ratio (Figure 7). Since S_1–*Eco*RI subfragments derived from the minor S_1 sites [such as one indicated by (●) in inset in Figure 6] are not protected at this ratio and the minor S_1 sites in sum account for the majority of total S_1 sensitivity, it is suggested that the B–Z junction is much more protected than the minor S_1 sites. On the other hand, the palindrome at residue 3065 is less protected than the B–Z junction but more protected than the minor S_1 sites (Figure 7); at a molar

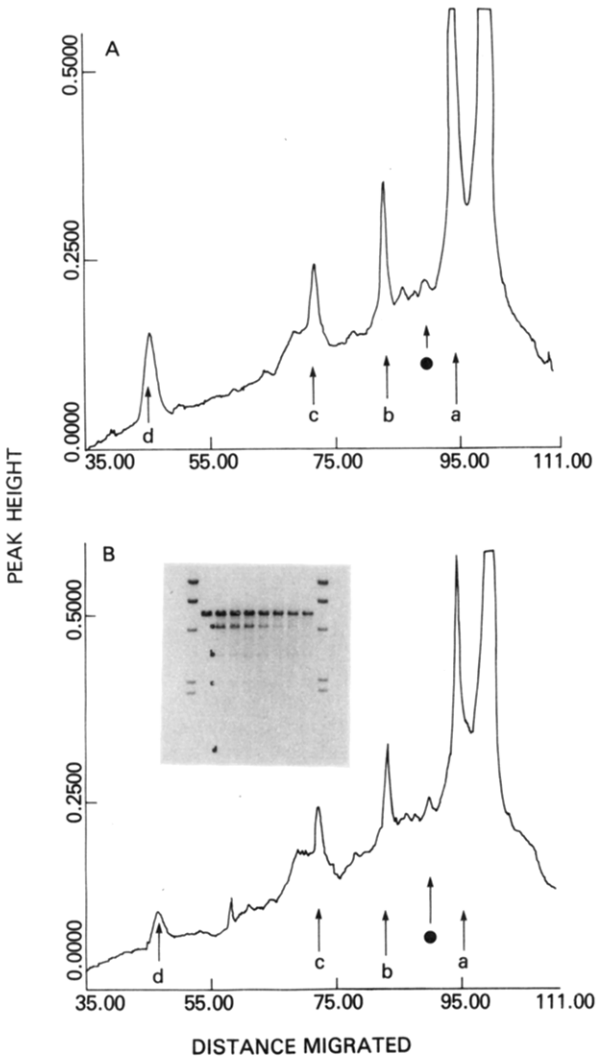

FIGURE 6: Specific protection of an S_1 site by HMG. Tracing of agarose gels of S_1 and *Eco*RI digests of pPst-0.9 in the presence of various amounts of HMG (see Materials and Methods). (A) HMG to DNA of 2; (B) HMG to DNA of 8. Inset in (B) (agarose gel): from left to right, molecular weight marker, linearized pPst-0.9, S_1 + *Eco*RI digests of pPst-0.9 at HMG to DNA ratio of 0, 2, 4, 8, 16, 32, and 64, molecular weight markers. Fragments a–d (see also Figure 1) were identified in both the gel and the scanner. (●) Minor S_1 cutting site.

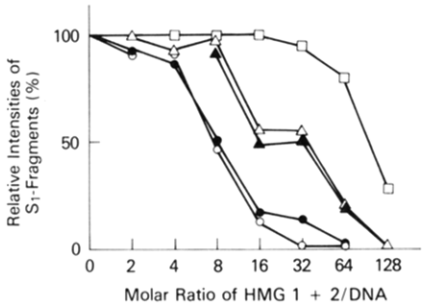

FIGURE 7: Relative protection of S_1 sites by HMG 1 and 2. The optical density of the various fragments generated from pPst-0.9 by S_1 and *Eco*RI in the absence of HMG is taken as 100%. Loss of a particular band indicates that S_1 did not cleave to produce such a band; i.e., the site is protected. (●), (○) 4.3- and 0.9-kb fragment (fragments a and d); (▲, △) 3.1- and 2.1-kb fragments (fragments b and c); (□) relative amount of linearized DNA estimated from Figure 5, lane 6–14, which represents total S_1 sites in pPst-0.9. The decrease in linear DNA with increasing amounts of HMGs is due to residual supercoiled DNA protected from S_1 digestion.

input of HMG 1 and 2 to DNA of 8, there is a preferential loss of bands a and d as compared to b and c (Figure 6). We

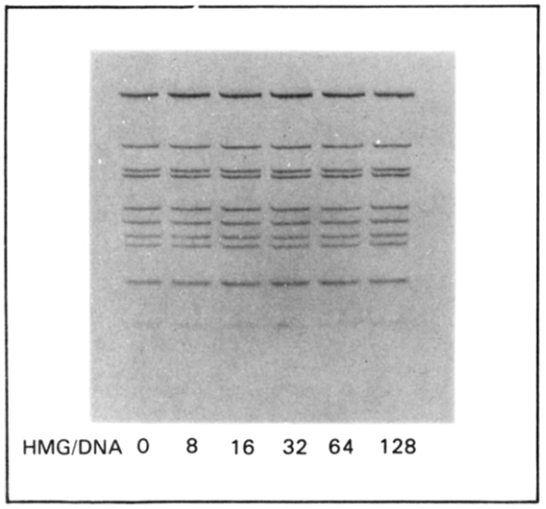

FIGURE 8: HMG does not protect pPst-0.9 from restriction digestion. Plasmid pPst-0.9 was incubated with HMG at the molar ratio indicated in 25 mM tris(hydroxymethyl)aminomethane (Tris) (pH 7.5)–10 mM $MgCl_2$–1 mM dithiothreitol (DTT) for 1 h at 4 °C. After incubation, the mixture was digested with *Hinf*I for 1 h at 7 °C and phenol extracted, and the recovered DNA fragments were electrophoresed in a 6% polyacrylamide gels.

interpret these results to mean that HMG 1 and 2 bind at or near the vicinity of the S_1 nuclease sensitive sites and that this binding protects the site from nuclease digestion. The binding is not random. Our results suggest a hierarchy of binding sites and imply that HMG 1 and 2 can distinguish between various S_1 nuclease sensitive sites in the plasmid.

Further evidence that the binding of HMG to the plasmid is not random was obtained by testing the ability of HMG to protect specific restriction sites in the plasmid. Obviously, protection of any restriction site would result in generation of new bands. The data presented in Figure 8 indicate that even at high amounts of HMG, where all S_1-sensitive sites are protected, all the restriction sites for enzyme *Hinf*I are digested. The DNA fragments produced by this enzyme in the absence of HMG are identical with those produced in the presence of various amounts of HMG. Identical results were obtained with restriction enzyme *Dde*I.

DISCUSSION

Previous findings demonstrated that chromosomal proteins HMG 1 and 2 can affect the superhelical structure of PM2 DNA (Javeherian et al., 1978) and that at 0.2 M NaCl these proteins display preferential binding to single-stranded DNA (Isackson et al., 1979). Our results confirm and extend these observations in that we report that HMG 1 and 2 can distinguish between superhelical and linear, double-stranded DNA. Furthermore, within the various possible binding sites present in one plasmid, the binding of this protein is not random. Plasmid pPst-0.9 contains three types of S_1-sensitive region: the major site at the B–Z junction, a prominent site at the palindrome centering at residue 3065 of pBR322, and a group of minor S_1 sites in pBR322, some of which have been mapped (Lilley, 1980; Panayotatos & Wells, 1981). Random binding of HMG to any of the possible sites would favor binding to the sum aggregate of the minor cutting sites, and preferential protection of the two major sites to S_1 cutting would not be observed. Our data however clearly indicate preferential protection in the following order: B–Z junction > palindrome at 3065 > minor S_1 sites. The data summarized in Figure 7 indicate that at an input molar ratio of HMG to DNA of 8, 50% of the B–Z junctions are protected while the S_1 digestion at position 3065 or at the many other minor S_1 sites is not affected. Thus, our results indicate that while HMG 1 and 2 display preference for single-stranded DNA, their binding to various sites with single-stranded properties is not random. The particular features responsible for preferential protection of the B–Z junction as compared to the palindrome at position 3065 and minor S_1 sites are not clear. In pPst-0.9, S_1 nuclease cleaves the B–Z junction at six prominent sites within a region of 20 base pairs (Hamada et al., 1984). The palindrome at position 3065 has three bases in the single-stranded loop (Panayotatos & Wells, 1981). It is possible that HMG preferentially binds to a longer single-stranded region. The possible relationships between S_1 susceptibility and cruciform formation in pBR322 are discussed elsewhere (Lilley, 1980; Panayotatos & Wells, 1981).

Since the binding of HMG to pPst-0.9 was inhibited both by single-stranded *E. coli* DNA and by plasmid pBR322, we concluded that the effects seen are not due to particular features of pPst-0.9 such as the eukaryotic insert of the human cardiac actin gene. The protection from the nuclease digestion is not due to enzyme inhibition since Reeck (Shasti et al., 1982) demonstrated that HMG 1 and 2 do not affect the activity of S_1 nuclease and provided evidence that the effects seen are due to the binding of HMG to DNA. It is also unlikely that the protection from S_1 nuclease confered by HMG 1 and 2 is simply due to nonspecific, random binding of HMGs to DNA. It has been shown that one molecule of HMG 1 unwinds the DNA by only 22–26° (Javaherian et al., 1979). We however have observed the preferntial protection of the B–Z junction at a HMG to DNA molar ratio of as low as 8. Even if all the input HMGs are bound to DNA, i.e., eight HMG molecules on one DNA molecule, the decrease in superhelical density would be only –0.001 [(24° × 8/360°)/500 (5000 bp/10 bp)]. Since the superhelical density of form I DNA used for our study is about –0.07, a change of –0.001 is not significant. Furthermore, the S_1 digestion of pPst-0.9 topoisomers with superhelical densities ranging between –0.06 and –0.08 showed that the BZ junction, the palindrome at 3065, and the minor S_1 site are all sensitive to S_1 nuclease (data not shown). Our results are in agreement with the data presented by Cockerill & Goodwin (1983), who noted that HMG proteins protect an S_1-sensitive site in the human globin gene from nuclease digestion. This site is within an alternating purine–pyrimidine region highly enriched in A-T residues. It is unlikely that such an A-T-rich region will adopt a Z-DNA conformation (Zimmerman, 1982; Jovin et al., 1983). Most likely, the S_1 sensitivity of this region and its subsequent protection by HMG reflect the general tendency of A-T-rich regions to adopt single-stranded conformation more readily than regions enriched in G-C residues.

The question arises whether the ability of HMG 1 and 2 to discriminate between various single-stranded regions in a plasmid reflects their cellular function. The ubiquitous distribution of HMG 1 and 2 suggest that they are involved in functions that are crucial to cell survival. The proteins are associated with chromatin; however, they are present also in the nucleoplasm and cytoplasm of the cells (Kleinschmidt et al., 1983; Bustin & Neihart, 1979; Einck et al., 1984). It has been suggested that the proteins shuttle between the nucleus and cytoplasm (Isackson et al., 1980) and that there is a correlation between cell proliferation and the cellular level of HMG 2 (Seyadin & Kistler, 1979). This correlation, however, may be due to difficulties in the extraction procedures of the proteins at different stages of the cell cycle (Shasti et al., 1982). Microinjection of antibodies into the nucleus of amphibian oocytes brought about cessation of transcription as evidenced

by retraction of transcription loops and by chromosome condensation (Kleinschmidt et al., 1983). In somatic cells, however, microinjection of anti-HMG 1 did not result in inhibtion of transcription (Einck & Bustin, 1983). Thus, at present it is not clear whether HMG 1 and 2 are merely structural proteins or are somehow involved in some way in affecting and regulting gene expression. Since S_1 sensitivity may be associated with gene transcription (Larson & Weintraub, 1982), it is possible that strategic placement of HMG would prevent or inhibit gene expression. It is clear however that the amount of HMG 1 and 2 present in a cell (approximately 1×10^6 molecules, (Johns, 1982) is such that the proteins cannot be associated with every single nucleosome in chromatin. Our results suggest that the protein can distinguish between various binding sites, and therefore, we assume that the binding of this protein in chromatin is not random. The ability of proteins HMG 1 and 2 to affect the structure of selected regions in the genome has obvious functional implications.

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