Substrate Structure Influences Binding of the Non-Histone Protein HMG-I(Y) to Free and Nucleosomal DNA†

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ABSTRACT: High mobility group protein HMG-I(Y) selectively binds to stretches of A·T-rich B-form DNA *in vitro* by recognition of substrate structure rather nucleotide sequence. Recognition of altered DNA structures has also been proposed to explain the preferential binding of this non-histone protein to four-way junction DNA as well as to restricted regions of DNA on random-sequence nucleosome core particles. Here we describe experiments that examine the influence of intrinsic DNA structure, and of structure imposed by folding of DNA around histone cores, on the binding of HMG-I(Y). As substrates for binding, we chose defined-sequence DNA molecules containing A·T-rich segments demonstrated previously to have very different structures in solution. These segments are either intrinsically bent (phased A·T tracts), flexible (oligo[d(A-T)]), or straight and rigid [oligo(dA)·oligo(dT)]. DNase-I and hydroxyl radical footprinting techniques were employed to analyze protein binding to these DNAs either free in solution or when they were reconstituted into monomer or dinucleosomes *in vitro*. Results indicate that the DNA structure exerts a significant influence on HMG-I(Y) binding both when substrates are free in solution and when they are wrapped into nucleosomal structures. For example, when DNA is free in solution, HMG-I(Y) prefers to bind to the narrow minor groove of A·T sequences but sometimes also binds to certain GpC residues having narrowed major grooves that are embedded in such sequences. On the other hand, depending on the structure and/or orientation assumed by particular A·T-rich segments on the surface of reconstituted histone octamers, HMG-I(Y) binding site selection on individual nucleosomes differs considerably. Two observations are of particular importance: (i) HMG-I(Y) can preferentially bind to certain types of A·T-DNA located on the surface of nucleosomes; and (ii) HMG-I(Y) binding can induce localized alterations in the helical periodicity and/or rotational setting of DNA on the surface of some nucleosomes. These abilities of HMG-I(Y) suggests that *in vivo* the protein may play an important role in recognizing and altering the structure of localized regions of chromatin.

Genetic and biochemical evidence demonstrates that chromatin structure exerts a dominant influence on the regulation of eukaryotic gene transcription *in vivo* [reviewed in Felsenfeld (1992), Paranjape et al. (1994), and Wolffe (1992)]. The presence of nucleosome core particles on critical *cis*-acting regulatory elements can repress transcription both *in vitro* and *in vivo* by preventing their recognition by *trans*-acting factors (Brown, 1984; Durrin et al., 1992; Lorch et al., 1992; Wolffe & Drew, 1989; Workman & Kingston, 1992). Numerous examples exist of precisely positioned nucleosomes playing an essential role in regulating transcription (Archer et al., 1991; Clark & Wolffe, 1991; Morse, 1989; Schild et al., 1993; Simpson, 1991; Svaren & Horz, 1993; Wolffe & Drew, 1989). In cases where inhibitory nucleosomes are positioned on key regulatory elements, the cell utilizes multiple mechanisms to render these sequences accessible to *trans*-acting factors in order to facilitate transcriptional activation. Some transcription factors, such as the glucocorticoid receptor (Perlman, 1992; Perlman & Wange, 1988; Pina et al., 1990) and GAL4 derivatives (Simpson, 1991; Workman & Buckman, 1993; Workman et al., 1991; Vettese-Dadey et al., 1994), possess DNA-binding motifs that allow binding to their recognition elements even when the DNA is wrapped around an unmodified histone octamer core. In these cases, both the rotational phasing and translational setting of the positioned nucleosome relative to the recognition element appear to be important for efficient factor binding, with accessibility for binding decreasing as the elements are moved from the ends toward the center of the core particle (Vettese-Dadey et al., 1994).

In other instances, exemplified by the binding of the *Xenopus* 5S RNA gene transcription factor TFIIIA, the composition of positioned nucleosome cores markedly influences factor access. For example, an unmodified histone octamer positioned on a 5S ribosomal gene represses transcription whereas a histone tetramer, $(H3/H4)_2$, located at the same site does not (Clark & Wolffe, 1991). Furthermore, *in vitro* binding of the 5S gene transcription factor TFIIA to nucleosome particles is enhanced when the amino-terminal tails of the core histones are either hyperacetylated or removed by protease treatment, suggesting a positive role for histone acetylation in facilitation of transcription factor access to DNA (Lee et al., 1993). *In vivo*, transcription

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factor binding can disrupt precisely positioned nucleosomes (Svaren & Horz, 1993; Morse, 1993). *In vitro*, however, nucleosome disruption following transcription factor binding is an ATP-dependent process (Tsukiyama et al., 1994) and, in certain instances, can be mediated by the multiprotein SWI/SNF "general" activator complex (Imbalzano et al., 1994; Kwon et al., 1994; Peterson & Herskowitz, 1992; Wolffe, 1994a; Yosihid et al., 1992). In other circumstances, the SWI/SNF complex may also stimulate the initial binding of transcription factors to nucleosomal DNA (Cote et al., 1994).

The HMG-I(Y)[1] family (Friedmann et al., 1993; Giancotti et al., 1991; Johnson et al., 1988, 1989) of "high mobility group" (HMG) mammalian proteins is a founding member of a recently described class of nuclear proteins collectively known as "architectural transcription factors" because of their ability to function *in vivo* both as components of chromatin structure and as ancillary gene transcription factors [reviewed in Bustin and Reeves (1996) Grosschedl et al. (1994), and Wolffe (1994b)]. In this capacity, the HMG-I(Y) proteins have recently been implicated in the *in vivo* transcriptional regulation of an increasingly large number of mammalian genes. In several examples of positive gene regulation, HMG-I(Y) is presumed to function by specifically interacting with other transcription factors (e.g., NF-κB, ATF-2, Elf-1, Oct-2, or Oct-6), as well as by bending or distorting promoter/enhancer DNAs sequences, in order to form a stereospecific multiprotein complex necessary for efficient transcription initiation [reviewed in Bustin and Reeves (1996)]. Mutations in an HMG-I(Y) gene family member have also been linked to the appearance of lipomas in humans (Ashar et al., 1995) and to the pygmy phenotype in mice (Zhou et al., 1995).

The HMG-I(Y) proteins are distinguished from other HMG proteins (Bustin & Reeves, 1995) by their ability to preferentially bind to the minor groove of the A·T-rich sequence of duplex DNA both *in vitro* (Elton et al., 1987; Radic et al., 1992; Reeves & Nissen, 1990; Reeves et al., 1987; Solomon et al., 1986) and *in vivo* (Disney et al., 1989; Saitoh & Laemmli, 1994). *In vitr*o binding of HMG-I(Y) bends and unwinds linear DNA substrates and induces supercoils into circular plasmid DNAs (Lehn et al., 1987; Nissen & Reeves, 1995). Even though HMG-I(Y) prefers binding to A·T-rich regions, it does not bind to all stretches of A·T-rich DNA equally well, or with equal affinity, and is influenced both by adjacent sequences and by the length and sequence of the A·T stretches, indicating that these proteins recognize the structure, rather than the sequence, of DNA (Reeves & Nissen, 1990). The structural binding preferences exhibited by HMG-I(Y) proteins became explicable with the elucidation of the regions of the proteins that interact with DNA (Reeves & Nissen, 1990). Individual proteins have three separate, but very similar, peptide domains (called A·T hook motifs) that specifically interact with the narrowed minor groove of A·T-DNA, and each of these binding domains is separated by a long and flexible polypeptide backbone that is predicted to undergo an induced conformational changed upon DNA binding (Reeves & Nissen, 1990). Recent two-dimensional solution ^{1}H NMR studies (Evans et al., 1992, 1995; Geierstanger et al.,1994) support the originally proposed model of the individual DNA-binding domains (Reeves & Nissen, 1990) that suggested that the peptide backbone of each of the DNA-binding regions has a planar crescent-shape structure resembling the drugs distamycin and netropsin, and the dye Hoechst 33258, ligands that also preferentially bind to the minor groove of A·T sequences.

In addition to its propensity to bind A·T-rich DNA, HMG-I(Y) also recognizes certain non-B-form structures found in supercoiled plasmids (Nissen & Reeves, 1995), synthetic four-way-junction DNAs (unpublished data; Claus et al., 1994), and restricted regions of DNA wrapped on the surface of random-sequence nucleosome core particles (Reeves & Nissen, 1993). The latter observations are of particular interest because they suggest that HMG-I(Y) binding to nucleosomes is, at least in part, due to recognition of altered DNA structures on the core particle surface (Reeves & Nissen, 1993). In the present paper, we report the result of experiments designed to test the hypothesis that the dominant factor governing HMG-I(Y) binding is the structure, not the sequence, of DNA. In our studies, we examined the influence of intrinsic DNA structure, and of structure imposed by folding of DNA into core particles, on the specific binding of HMG-I(Y). As substrates for HMG-I(Y) binding, we chose defined-sequence DNA molecules containing A·T-rich segments demonstrated previously to have different structures in solution. DNase-I and hydroxyl radical cleavage techniques were employed to analyze HMG-I(Y) protein binding to these DNAs either free in solution or after they had been reconstituted *in vitro* into monomer or dinucleosomes. Our results clearly indicate that HMG-I(Y) binding is sensitive to DNA structure, preferring to bind to stretches of DNA with narrowed grooves when the substrate is free in solution but altering its sites of recognition when the same DNA is deformed by wrapping into nucleosomal particles. Of particular note are the findings that in certain instances HMG-I(Y) binding appears to change the localized helical periodicity and/or rotational setting of DNA on core particles and in other cases the protein is able to bind to stretches of A·T-DNA located on the nucleosome surface. These observations suggest a possible role for HMG-I(Y) proteins in the recognition and remodeling of localized DNA and chromatin structures *in vivo*.

MATERIALS AND METHODS

DNA Fragments. Derivatives of plasmid T7CAT containing ~50 bp segments of "structured DNA" (curved, flexible, or rigid) inserted into a unique *Bam*HI site upstream of a T7 polymerase promoter (Figure 1) have been previously described (Wolffe & Drew, 1989; Hayes et al., 1991b). Plasmid DNAs were purified by QIAGEN column chromatography (QIAGEN Inc., Chatsworth, CA) and manipulated by standard techniques (Ausubel et al., 1988). Restriction enzyme digestion fragments of either 271 bp (*Hind*III−*Pvu*II) or 147 bp (*Hind*III−*Dde*I) length were 5′-radiolabeled at the *Hind*III site (Figure 1) and used for in vitro nucleosome reconstitutions. Specifically, plasmids were first cut with *Hind*III, treated with alkaline phosphatase, and then radiolabeled with T4 polynucleotide kinase and [γ-^{32}P]ATP before subsequent restriction with either *Pvu*II or *Dde*I. The labeled

[1] bp, base pair(s); HMG, high mobility group nonhistone chromatin proteins; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; nt, nucleotide; HMG-I(Y), recombinant human HMG-I protein; RP-HPLC, reverse-phase high-performance liquid chromatography; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

fragments were separated by electrophoresis on 3.5% agarose gels and isolated from gel fragments either by electroelution or by QIAEX (QIAGEN Inc.) column chromatography.

Purification of HMG Proteins. Full-length recombinant human HMG-I protein [hereafter called HMG-I(Y) to distinguish it from the unrelated HMG-1 family of proteins; Bustin & Reeves, 1996] was produced using the expression vector pET7C carrying the full-length human HMG-I cDNAs as previously described (Johnson et al., 1989). The purity of each preparation was assessed by SDS−polyacrylamide gel electrophoresis (SDS−PAGE) (Ausubel et al., 1988). HMG-I(Y) protein concentration was determined spectrophotometrically using $\epsilon_{220} = 74\,000$ L/(mol·cm) (Reeves & Nissen, 1990). Native HMG-17 protein from murine ascites cells was isolated and quantified as previously described (Elton & Reeves, 1986).

Nucleosome Core Particles and Histone Octamers. Adult whole chicken blood in sodium citrate was purchased from Pel-Freez (Rogers, AK). Trimmed chicken erythrocyte core particles were prepared by a modification of the methods described by Libertini and Small (1980) as previously described (Reeves & Nissen, 1990). The trimmed core particles were purified by gel filtration on a column of Sepharose CL-6B. Core particle preparations were routinely found to predominantly (>95%) contain DNA of ~146 bp. Analysis of the protein content of core particle preparations by SDS−PAGE revealed the nucleosome cores to be essentially devoid of H5/H1 histones and non-histone HMG proteins and to contain >95% undegraded core histones. Histones H2A/H2B and H3/H4 were purified from isolated core particles by hydroxyapatite chromatography (Simon & Felsenfeld, 1979), concentrations determined using $\epsilon_{230} = 4.2$ L/(g·cm) (Stein, 1979), and monitored by denaturing gel electrophoresis.

Chromatin Reconstitutions. Monomer and dinucleosomes were reconstituted onto radiolabeled DNA fragments either by exchange with core particles (Tatchell & van Holde, 1977) or by dialysis from high salt/urea with purified histones (Camerini-Otero et al., 1976; Simon & Felsenfeld, 1979). Quality control of chromatin reconstitutions was monitored by native nucleoprotein gel electrophoresis (0.8% agarose, 45 mM Tris−borate, pH 8.3, and 1 mM EDTA) and the integrity of the core histones checked before and after reconstitutions by denaturing SDS−PAGE gel electrophoresis (Wolffe & Hayes, 1993). Typically, for both mononucleosome and dinucleosome preparations, less than 5% of the input radiolabeled DNA fragments were present after the reconstitutions as either free DNA or other types of unwanted reconstitution products.

DNase-I and Hydroxyl Radical Cleavage. Reconstituted monomer or dinucleosomes were incubated with recombinant HMG-I(Y) (or, in some cases as a control, native HMG-17) as previously reported (Reeves & Nissen, 1993) at the various HMG:core particle molar ratios described in the text. The resulting HMG−nucleosome complexes were cleaved with either DNase-I or the hydroxyl radical following published protocols (Tullius et al., 1987; Wolffe & Hayes, 1993). The integrity of the reconstituted chromatin particles was not affected by exposure to either cleavage reagent as monitored by nucleoprotein gel electrophoresis. The optimum cleavage conditions for each reagent and for each reconstituted chromatin preparation were determined empirically. After treatment, chromatin particles were immediately isolated from cleavage reagents, free DNA, and any other contaminating materials by electrophoresis on 0.8% agarose (45 mM Tris−borate, pH 8.3, 1 mM EDTA) native nucleoprotein gels. The electrophoretic positions of the reacted monomer or dimer nucleosomes were identified by autoradiography, the appropriate gel fragments excised, and the chromatin particles electroeluted. Labeled DNA fragments were recovered from the chromatin particles by protease digestion and phenol/chloroform extraction (1:1, v/v) and precipitated with ethanol (Wolffe & Hayes, 1993). Single-stranded DNA cleavage products were then separated by electrophoresis on either 6% or 8% sequencing gels with Maxam−Gilbert "G-lane" chemical cleavage products of control DNA fragments serving as reference standards (Ausubel et al., 1988). Autoradiograms of the sequencing gels were analyzed on a Molecular Dynamics computing densitometer with ImageQuant and Microsoft Excel 4.0 software. Fourier transformations and other data analyses were performed using SigmaPlot and PeakFit software programs from Jandel Scientific (Corte Madera, CA). The helical periodicities of nucleosomal DNAs were determined by the hydroxyl radical cleavage methods and gel quantitation procedures previously described (Hayes et al., 1990, 1991b). Briefly, the area of each cleavage band was determined by integration using the ImageQuant program, and integrals of bands from the control (free DNA) samples were subtracted from the integrals of the corresponding bands in the nucleosome samples. The resulting values, which represent the amount of cleavage at each nucleotide in the nucleosome, were then smoothed and analyzed using the curve-fitting programs of PeakFit.

RESULTS

HMG-I(Y) Binding to Structured DNAs. In order to investigate the influence of substrate structure, as opposed to sequence, on the recognition and binding of non-histone protein HMG-I(Y) to DNA we followed the strategy of characterizing, by DNase-I and hydroxyl radical cleavage techniques, the footprinting and cleavage patterns of the protein on a number of different "structured" DNAs before, and after, they were reconstituted into nucleosome particles *in vitro*. The rationale behind this approach is that if structure is the predominant factor determining HMG-I(Y) binding, then the protein should recognize different sites on a given DNA fragment when it is free in solution compared to when it is bent and distorted around a nucleosome histone core. The reason for this is that DNA that is wound on the surface of core particles deviates considerably from B-form and is not uniformly bent but contains localized regions that are severely deformed or kinked (Richmond et al., 1984; Hayes et al., 1990, 1991a,b). Figure 1 diagrams derivatives of the T7CAT vector that contain the different A·T-rich "structured" DNA fragments used either as naked DNA or after association with core histones. All of these A·T-containing fragments have previously been analyzed by both DNase-I and hydroxyl radical cleavage reactions (Wolffe & Drew, 1989; Hayes et al., 1991b) and shown to have quite different structures in solution, but very similar structures once they have associated with histones as nucleosomal particles (Hayes et al., 1991b). Two of the fragments, UL1 and UL4, are intrinsically bent and contain short (6 bp) A·T tracts every 10−11 bp separated by GGCC/CCGG base pairs

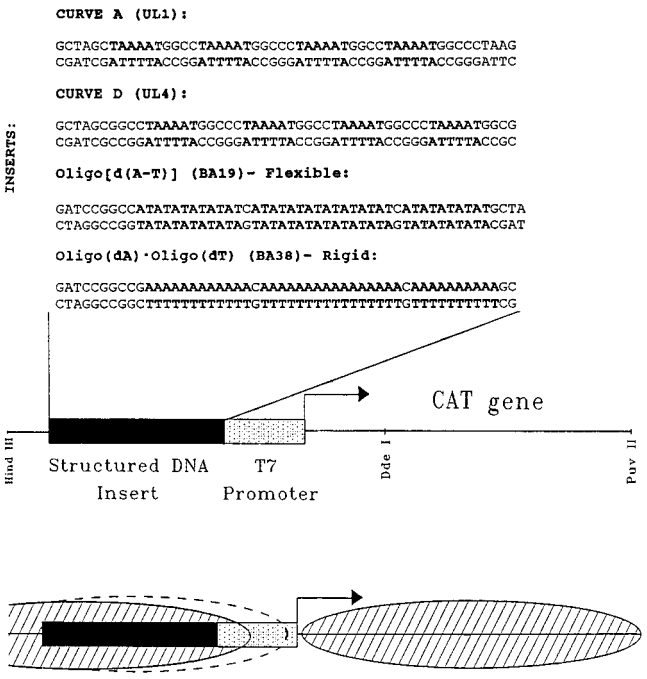

FIGURE 1: Design and sequence of "structured" DNA fragments (Wolffe & Drew, 1989). The four DNA fragments (52-mers) of known sequence and structure (black box) were inserted into the pT7CAT plasmid at a *Bam*HI site immediately upstream from the T7 promoter (stippled box) adjacent to the bacterial chloramphenicol acetyltransferase (CAT) gene. Isolated fragments, radiolabeled at the *Hind*III site, were used in chromatin reconstitution and footprinting experiments: 271 bp *Hind*III−*Pvu*II fragments were reconstituted into dinucleosomes, and 142 bp *Hind*III−*Dde*I fragments were assembled into monomer nucleosomes. The approximate positions of the dinucleosomes reconstituted on the 271 bp *Hind*III−*Pvu*II UL1 and UL4 fragments after *in vitro* reconstitutions [as identified by Wolffe and Drew (1989)] are shown diagrammatically by the hatched ovals in the lower part of the figure. The position of the nucleosome located over the CAT gene is the same in both reconstitutes whereas the solid oval over the "structured" DNA shows the approximate position of the second nucleosome in the UL4 reconstitutes and the dashed oval indicates the nucleosome position in the UL1 reconstitutes.

and are similar in sequence to the naturally curved kinetoplast DNAs. In such repetitively spaced sequences, curvature is thought to result from the fact that in solution the highly bent GpC steps have a marked tendency to lie with their relatively wide minor grooves along the convex side of curved DNA, whereas the oppositely bent ApA steps prefer to lie with their relatively narrow minor grooves along the concave side of curves (Satchwell et al., 1986; Travers, 1989). Electrophoretic studies have demonstrated that AAAAA and GGGCCC sequences curve DNA in opposite directions by the same amount (Brukner et al., 1993). In the case of curves UL1 and UL4 (Figure 1), the GpC and ApA steps are out of phase with each other by half a helical turn of DNA, thus maximizing curvature of the fragments. The predicted periodic narrowing and widening of minor groove width is easily detected by hydroxyl radical cleavage of kinetoplast DNA (Burkhoff & Tullius, 1987; Hayes et al., 1991b) and is also readily apparent in the curved constructs employed here (e.g., see Figures 5 and 7A). In vitro nucleosome reconstitution experiments have shown that the histone core exhibits a marked preference for association with DNAs, such as UL1 and UL4, that have an intrinsic curvature or anisotropic flexibility with a periodicity of ~10.0 bp/turn (Schrader & Crothers, 1990). In such nucleosome reconstitutes, the concave side of the DNA curve with its narrowed grooves is, as predicted (Travers, 1989; Schrader & Crothers, 1990), oriented toward the histone core surface (Hayes et al., 1991b). Curves UL1 and UL4 differ by 6 bp in the distance of their first A·T tract from the T7 RNA polymerase promoter and, as shown diagrammatically by the solid and dashed ovals in the lower part of Figure 1, this difference influences the positioning of the dinucleosomes formed on the UL1 and UL4 DNA fragments during *in vitro* reconstitutions (Wolffe & Drew, 1989).

We also made use of flexible and rigid DNAs in our experiments (Figure 1). For example, DNA fragment BA38 contains long homopolymeric A·T stretches [e.g., oligo(dA)·oligo(dT)] that are predicted to be relatively straight and rigid in solution with a decidedly narrowed minor groove (Nelson et al., 1987; Coll et al., 1987). These homopolymeric structures are overwound with respect to normal B-form DNA having a helical periodicity of approximately 10.0 bp/turn (Rhodes & Klug, 1981) and have base pairs with large propeller twists and an ordered water structure in the narrow minor groove (Drew & Dickerson, 1981; Fratini et al., 1982; Nelson et al., 1987; Coll et al., 1987). In contrast, the DNA fragment BA19 contains tracts of alternating A/T residues (e.g., oligo[d(A-T)]) that are predicted to be conformationally flexible in solution as a consequence of the instability of TpA dinucleotide steps (Drew & Dickerson, 1981; Travers & Klug, 1987). Theoretical considerations (Calladine, 1982; Chupina, 1985) suggest that a TpA step is incompatible with high propeller base twist, thus forcing these dinucleotides to have a widened minor groove.

Figure 2 shows the DNase-I cleavage and protection pattern of HMG-I(Y) on the free DNA of a representative of each type of "structured" fragment investigated: curved (UL4), rigid (BA38), and flexible (BA19). In each case, the substrate was a 271 bp long *Hind*III−*Pvu*II restriction fragment 5′ end-labeled at the *Hind*III site (Figure 1). The results indicate that, consistent with earlier reports, HMG-I(Y) preferentially binds to the A·T-rich regions of all of these types of structured DNA fragments. It is also apparent, however, that there are interesting and reproducible variations in HMG-I(Y) binding between the different structured DNAs. For example, there are significant differences in the affinity of HMG-I(Y) binding to the different types of substrates. Thus, considering the large number of potential A·T-binding sites present in each of these fragments (Reeves & Nissen, 1990), HMG-I(Y) readily footprints at relatively low concentrations [molar ratios of protein to DNA of (10−15):1] to the curved UL4 and rigid BA38 DNAs with the most complete nuclease protection occurring on the rigid molecule with its considerably narrowed minor groove (Figure 2, BA38, lanes 3 and 4). In contrast, a much higher molar ratio of HMG-I(Y) to DNA [e.g., (50−60):1] is required for footprinting to the alternating A·T residues present in the BA19 DNA, and even at this high protein concentration, the footprint over the A·T regions of the BA19 fragment is not complete (Figure 2; BA19, lane 3). These results indicate that the protein binds less efficiently to flexible alternating A·T sequences than to either the rigid or the curved DNAs. This difference in binding affinity is understandable since the TpA steps in the BA19 DNA are predicted to have a wide minor groove (Calladine, 1982) whereas HMG-I(Y) prefers binding to A·T sequences with narrowed minor

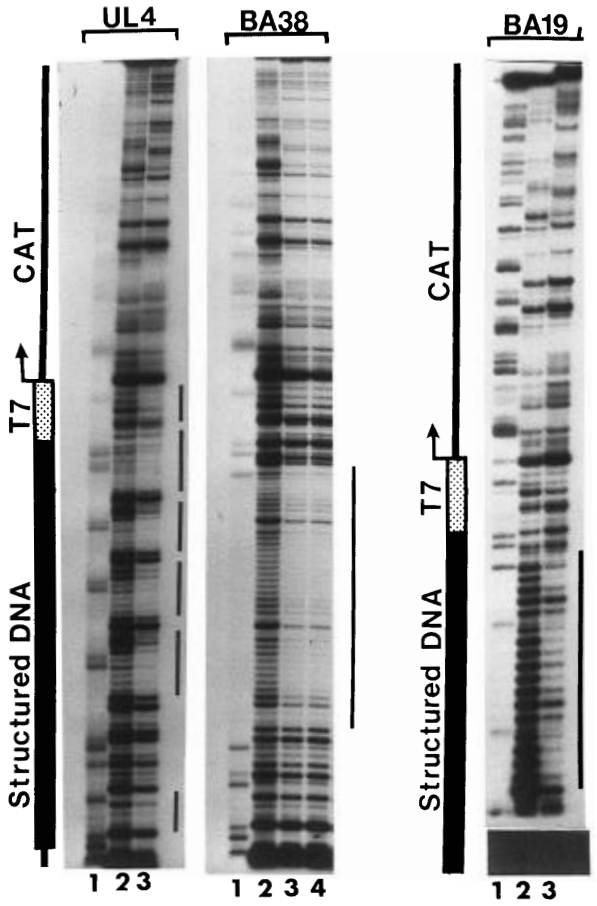

FIGURE 2: DNase-I footprints of HMG-I(Y) on structured DNAs free in solution. 5′ end labeled, 271 bp long *Hind*III−*Pvu*II fragments of either curved UL4, rigid BA38 [oligo(dA)·oligo(dT)] or flexible BA19 (oligo[d(A-T)]) DNAs were reacted with HMG-I(Y) at a 25:1 protein:DNA molar ratio (except for BA19, which was at a 60:1 molar ratio) and then digested with DNase-I as described under Materials and Methods. In each case, lane 1 is a Maxam−Gilbert G-reaction marker, lane 2 is free DNA digested with DNase-I, and lane 3 is DNA plus HMG-I(Y) digested with DNase-I. lane 4 in the BA38 group is a DNase-I digest of a sample with a protein:DNA molar ratio of 15:1. Solid vertical lines to the right of each panel indicate areas of protection from DNase-I digestion by HMG-I(Y). At the left of the figures are diagrams of the arrangement and location of the important sequences for each of the DNA restriction fragments (coding as in Figure 1).

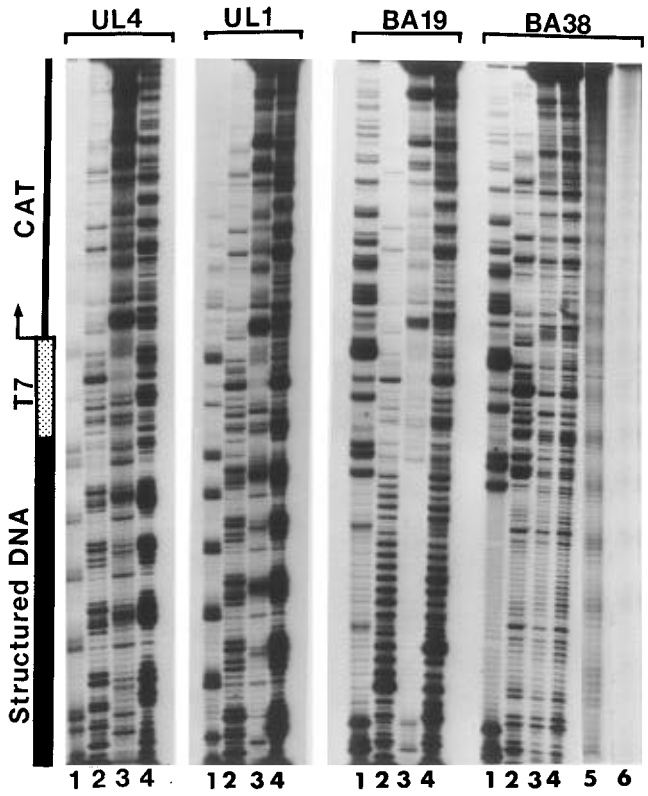

FIGURE 3: DNase-I and hydroxyl radical cleavages of reconstituted dinucleosomes. In each case, the 5′-end-labeled 271 bp "structured" DNA restriction fragments were reconstituted into dinucleosomes and digested with DNase-I for either 2 min (lane 3) or 4 min (lane 4). Lane 1, Maxam−Gilbert G-reaction marker; lane 2, free DNA digested with DNase-I. lanes 5 and 6 of the BA38 sample show hydroxyl radical cleavage patterns of the reconstituted dinucleosome and the free DNA, respectively. At the far left is a diagram of the arrangement of the important sequences in the DNA restriction fragments (as in Figure 1).

grooves (Reeves & Nissen, 1990) such as those present in the curved and rigid DNAs.

Of perhaps greater interest, the DNase-I digestion results in Figure 2 also demonstrate that HMG-I(Y) binds to the curved UL4 DNA in a telling, and somewhat novel, manner. In this case, HMG-I(Y) is observed not only to footprint to stretches of A·T sequence but also to protect certain G·C base pairs embedded within such sequences. Similar HMG-I(Y) footprinting is also observed with the curved UL1 DNA (data not shown) except that in this case the protection pattern is offset by 6 bp in the two different fragments, indicative of the fact that this is the relative distance of the first A·T tract of each of the curves from the T7 promoter (Figure 1). The ability of HMG-I(Y) to protect G·C residues that are flanked by A·T regions in both the UL1 and UL4 fragments most likely reflects recognition by the protein of the intrinsic structure of these curved sequences. GpC steps are highly bent in many crystalline forms of DNA, and the sequence GGCC/CCGG is known to be curved with about a 20° closing of the major groove and an opening of the minor groove (Goodsell et al., 1993). The narrowed major grooves of these GpC steps have a tendency to lie along the same concave side of the curves as do the narrowed minor grooves of the flanking A·T sequences (Satchwell et al., 1986; Travers, 1989) and, thus, are also likely to form suitably structured substrates for HMG-I(Y) binding. Whatever the structural features recognized by the protein, as mentioned previously, the affinity and specificity of HMG-I(Y) binding are significantly influenced by both the length and the sequence of particular A·T stretches, as well as by the "context" of flanking or adjacent nucleotides (Elton et al., 1987; Reeves & Nissen, 1990; Radic et al., 1992; Reeves et al., 1987; Russnak et al., 1988; Solomon et al., 1986; Skalnik & Neufeld, 1992). In particular, the present observations are consistent with earlier footprinting data demonstrating that G·C residues embedded in stretches of A·T richness can often be protected by HMG-I(Y) binding (Radic et al., 1992; Reeves et al., 1987; Russnak et al., 1988; Skalnik & Neufeld, 1992). Importantly, the present results lend considerable additional support to the notion that it is the structure, rather than the sequence, of the substrate that plays a dominant role in the selectivity and binding of HMG-I(Y) to DNA.

Reconstitution of Dinucleosomes. Figure 3 shows the results of experiments in which the different 5′ end-labeled *Hind*III−*Pvu*II DNA restriction fragments were reconstituted *in vitro* with core histones and the structure of the resulting

chromatin analyzed by DNase-I digestions. As previously described (Wolffe & Drew, 1989; Pruss et al., 1994), the ~10 bp periodicity of DNase-I cleavage and hydroxyl radical cleavage patterns observed in the reconstitutes indicate that the 271 bp of DNA in these fragments is associated with two histone octamers per molecule. Each of these fragments contains the bacterial CAT (chloramphenicol acetyltransferase) gene, which strongly positions a histone octamer with one boundary at the edge of the gene sequence (Wolffe & Drew, 1989; Izban & Luse, 1991; Pruss et al., 1994). The other histone octamer lies over the T7 promoter and the segment of structured DNA (Figure 1). The exact position of this second histone octamer is dependent on the sequence of "structured" DNA (Wolffe & Drew, 1989; Pruss et al., 1994) (Figure 1). Histone−DNA contacts within the regions of structured DNA in the second nucleosome have been reported (Wolffe & Drew, 1989; Pruss et al., 1994) to begin and end at different DNA sequences (translational positioning) and to have different DNA sequences facing toward the histones or the solution (rotational positioning) in the different reconstitutes.

In preliminary experiments, we confirmed that the reconstitutions of these DNA fragments into nucleosomal complexes were identical to those that we have previously described (Wolffe & Drew, 1989; Hayes et al., 199b; Pruss et al., 1994) by conducting a series of nuclease digestion experiments employing various restriction enzymes, micrococcal nuclease, and DNase-I. DNase-I footprinting reveals very similar cleavage patterns over the CAT gene region in all of the nucleosomal templates (cf. lanes 4 in Figure 3, downstream of the *hooked arrow*), but variant patterns over the segments of both structured DNA (Figure 3, solid box) and the T7 promoter (Figure 3, stippled box). Although the repetitive ~10 bp DNase-I cleavage pattern characteristic of nucleosomal DNA is apparent over the region of structured DNA in the other DNA fragments, in the reconstituted BA38 DNA this repetitive cleavage pattern is less distinct over the rigid homopolymeric segment (Figure 3, BA38, lane 4). This observation is consistent with the report of Hayes et al. (1991b), who demonstrated that rigid oligo(dA)·oligo(dT) segments can be reconstituted into nucleosomes although such distortion is energetically unfavorable. Furthermore, the nucleosomal distortion is not easily revealed by DNase-I digestion since this large and bulky enzyme does not favor oligo(dA)·oligo(dT) as a substrate (Drew, 1984), but is observable when the nucleoprotein complex is cleaved by the much smaller hydroxyl radical molecule (Hayes et al., 1991b) as shown in Figure 3 (BA38, lane 5). Together, the results in Figure 3 confirm the previously reported strong positioning, both translational and rotational, of one nucleosome over the CAT gene and the variant positioning of the second nucleosome over the T7 promoter and structured DNAs in these reconstituted dinucleosomes (Wolffe & Drew, 1989; Pruss et al., 1994).

HMG-I(Y) Binding to Dinucleosomes. In competition experiments, HMG-I(Y) preferentially binds to free, rather than nucleosomal, DNA (Reeves & Nissen, 1993; unpublished observations). In all of the reconstitution and cleavage experiments, it was therefore necessary, after the *in vitro* reactions, to electrophoretically separate the HMG-I(Y)-bound nucleosomes from free DNA and from any unbound or partially bound particles or other contaminants and use these purified particles for subsequent analyses. Figures 4

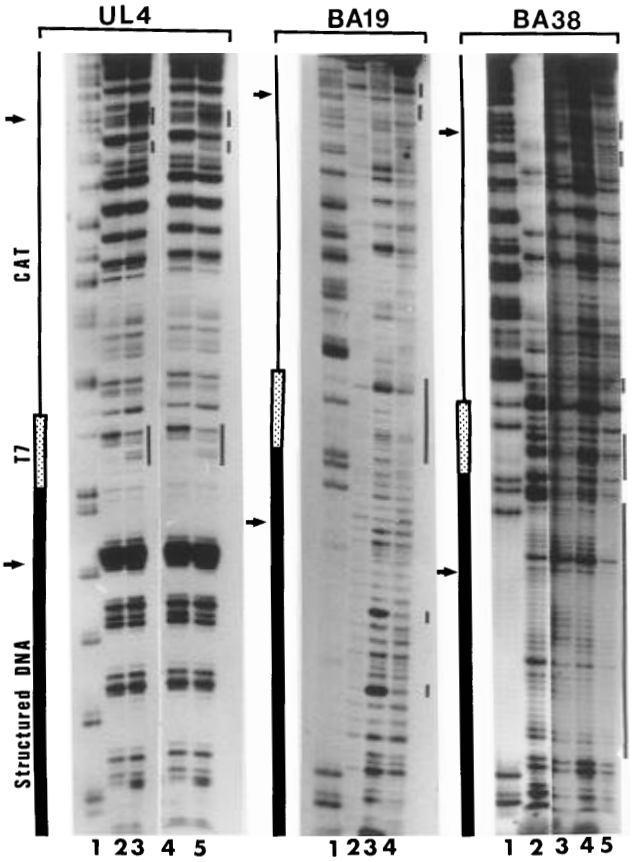

FIGURE 4: DNase-I cleavage patterns of UL4, BA19, and BA38 dinucleosomes without, and with, bound HMG-I(Y) . Recombinant HMG-I(Y) protein was added to reconstituted dinucleosomes at the ratios indicated, cleaved with DNase-I, and subsequently processed as described under Materials and Methods and in the text. Panel UL4; Lane 1, Maxam−Gilbert G-reaction marker; lane 2, dinucleosomes without HMG-I(Y); lane 3, dinucleosomes plus HMG-I(Y) at a molar ratio of 8:1, protein:DNA; lane 4, dinucleosomes without HMG-I(Y); lane 5, dinucleosomes plus HMG-I(Y) at a molar ratio of 4:1, protein:DNA. Companion lanes 2 and 3 and 4 and 5 are from independent experiments. Panel BA19: Lane 1, Maxam−Gilbert G-reaction marker; lane 2, free DNA; lane 3, dinucleosome without HMG-I(Y); lane 4, dinucleosome plus HMG-I(Y) at a molar ratio of 8:1, protein:DNA. Panel BA38: Lane 1, Maxam−Gilbert G-reaction marker; lane 2, free DNA; lane 3, dinucleosome without HMG-I(Y); lane 4, dinucleosome plus HMG-I(Y) at a 4:1 protein:DNA ratio; lane 5, dinucleosome plus HMG-I(Y) at a molar ratio of 10:1, protein:DNA. It should be noted that there is a slight overloading of the sample in lane 4 compared to those in lanes 3 and 5. Therefore, a more accurate assessment of the differences between the DNase-I cleavage patterns present in HMG-I(Y)-bound and unbound BA38 dinucleosomes is obtained by comparing the cleavage patterns in lanes 3 and 5. Laser densitometry scans of lanes 3 and 5 confirm the differences in cleavage patterns between bound and unbound dinucleosomes described in the text (data not shown). To the left of each panel in this Figure is a diagram of the important features of the DNA fragment with the arrows indicating the putative dyad axes of each of the nucleosomes. The vertical solid lines to the right of the lanes indicate areas where significant changes occur upon HMG-I(Y) binding.

and 5 show DNase-I and hydroxyl radical cleavages, respectively, of reconstituted dinucleosomes with, and without, bound HMG-I(Y). From these figures, it is evident that assembly of the 271 bp fragments into dinucleosomes leads to major changes in the patterns of HMG-I(Y) binding and cleavage on the reconstituted fragments compared to those seen on the same DNAs in free solution (cf. Figure 2). It is also evident that each of the individual dinucleosomes has

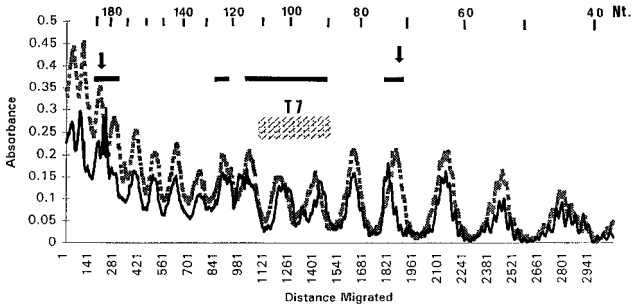

FIGURE 5: Hydroxyl radical cleavage of UL1 dinucleosomes without, and with, bound HMG-I(Y). Laser densitometry scan of dinucleosomes without (solid line) or with bound HMG-I(Y) at a molar ratio of 4:1, protein:DNA (broken line). Distance in nucleotides (Nt) from the 5′ end-labeled *Hind*III site is show across the top of the figure. The position of the T7 promoter (stippled box) and the putative positions of the dyad axes of each of the nucleosomes (arrows) are indicated. The horizontal solid lines indicate areas of the gel showing significant changes as a result of HMG-I(Y) binding.

localized regions with significantly different cleavage patterns when HMG-I(Y) is bound than when it is not. The solid lines beside each of the lanes with added HMG-I(Y) in Figure 4 (and above the scans in Figure 5) indicate areas where significant differences in cleavage patterns are observed upon protein binding to dinucleosomes. Although, as described below, the different classes of reconstituted DNA fragments show considerable individuality of cleavage patterns in restricted areas upon HMG-I(Y) binding, interestingly there are also some common cleavage patterns observed in all of the different HMG-bound dinucleosomes. For example, in all of these HMG-I(Y)-bound dimers, there are altered patterns and enhanced cleavages [detectable by both DNase-I (Figure 4) and hydroxyl radicals (Figure 5)] around the putative dyad axis of the nucleosome that is strongly positioned over the CAT gene. Additionally, as has previously been reported for isolated native nucleosome particles (Reeves & Nissen, 1993), all of the reconstituted nucleosomes employed in this study also showed significant HMG-I(Y) binding to the DNA at the entrance and/or exit of the reconstitutes (data not shown).

Individual HMG-I(Y)-bound dinucleosomes also exhibit significant alterations in their cleavage patterns in localized areas depending on their type of structured DNA. For example, although there are minor changes observable throughout the dinucleosomes, perhaps the most striking feature of both the UL4 (Figure 4; compare lanes 2 and 3 and 4 and 5) and UL1 (data not shown; also see Figure 5) reconstitutes is that when these intrinsically curved DNAs are wrapped about the surface of histone octamers the HMG-I(Y) protein no longer shows the same degree of strong preferential binding to the alternating tracts of phased A·T and G·C residues present in the "structured" regions of the DNAs as it does when these same fragments are free in solution (cf. Figure 2). The likely explanation for this unexpected situation is that in the reconstituted nucleosomes the concave sides of their curved DNAs have their narrower minor and major grooves (see above) oriented toward the histone core surface (Travers, 1989; Schrader & Crothers, 1990; Hayes et al., 1991b), thus precluding HMG-I(Y) from binding to these preferred regions (see also Figures 5, 6, and 7A).

Another unexpected and characteristic effect of HMG-I(Y) binding to the curved UL1 and UL4 dinucleosomes is a marked alteration in the cleavage patterns seen in and around the T7 promoter. As shown by the DNase-I cleavage patterns of reconstituted UL4 DNA shown in Figure 4 (vertical bars next to lanes 3 and 5), the most obvious of these changes is the appearance of two prominent new, HMG-I(Y)-induced, cleavage bands within the T7 promoter sequence (compare lanes 2 and 3 and lanes 4 and 5). This alteration of nuclease digestion pattern suggests that an HMG-I(Y)-induced structural change (Reeves & Nissen, 1993; Nissen & Reeves, 1995) has occurred within the nucleosome DNA that is recognized and selectively cleaved by DNase-I (also see Figure 6, below). However, due to its large size and hindrances that prevent it from attacking with equal efficiency at all sites at an angle normal to the core particle surface (Klug & Lutter, 1981), the DNase-I enzyme has only limited usefulness as a probe for changes in nucleosomal DNA structure. Therefore, to obtain a more refined picture of these HMG-I(Y)-induced alterations, we employed the hydroxyl radical (Burkhoff & Tullius, 1987; Tullius et al., 1987) as a probe of DNA structure. A major advantage of this method is that, due to its small size and lack of sequence specificity, hydroxyl radical cleavage is an extremely sensitive probe of DNA structure and has been successfully used to detect changes in the structure and helical periodicity of DNA within nucleosomes at single-nucleotide resolution (Hayes et al., 1990; 1991a,b). Figure 5 shows the laser densitometry scans of hydroxyl radical cleaved DNA isolated from UL1 dinucleosomes without (solid line) or with (broken line) bound HMG-I(Y) protein, and it is evident that, as with nuclease digestions, marked alterations in and around the T7 promoter area are also observed with this reagent. In this case, however, rather than the appearance of entirely new cleavage bands, fragmentation by the much smaller hydroxyl radical reveals what appears to be a marked HMG-I(Y)-induced shift in the position of the major DNA cleavage products in the region of the T7 promoter in UL1 dinucleosomes. As will be demonstrated below, this localized alteration in hydroxyl radical cleavage pattern is the result of an apparent HMG-I(Y)-induced perturbation in the helical periodicity of this nucleosomal DNA.

Figure 4 also illustrates that individuality of cleavage patterns is likewise observed in the structured A·T-DNA regions of protein-bound BA19 and BA38 dimers. For example, although there are minor observable differences distributed throughout the CAT region, in the HMG-I(Y)-bound BA19 dinucleosome, strong DNase-I protections are seen in two localized areas of the flexible oligo[d(A-T)] stretch of DNA (short vertical bars next to lane 3 of the BA19 panel in Figure 4) and in a larger area in and around the T7 promoter (long vertical bar). By contrast, it is apparent even by visual inspection (and further confirmed by laser densitometry; data not shown) that HMG-I(Y)-bound BA38 dinucleosomes (lanes 4 and 5 of Figure 4), when compared with unbound dinucleosomes (lane 3), show noticeable changes in, or protections against, DNase-I cleavage in the areas around the dyad axis of the positioned CAT nucleosome and in the region of the T7 promoter as well as over much of the extended oligo(dA)·oligo(dT) tract. In particular, this DNase-I footprinting pattern over the oligo(dA)·oligo(dT) tract was initially unexpected since the HMG-I(Y)-protected region on the BA38 dimer stretches for about 40 bp and is thus located on the lateral surface of the second

nucleosome and extends past its dyad axis (also, see below). Importantly, the nucleosomal cleavage patterns located immediately adjacent on either side of this protected region (except for the T7 promoter) are very similar in the BA38 dimers whether or not they contain bound HMG-I(Y). As noted previously, all of the HMG-I(Y)-bound nucleosome particles used in these studies were isolated by native agarose electrophoresis immediately following experimental digestions, and thus spurious contaminants are unlikely to have contributed to the observed protein-binding results. Control experiments also indicated that HMG-I(Y) binding does not lead to detectable nucleosome dissociation under the low ionic strength electrophoretic conditions used in the assays (data not shown).

HMG-I(Y) Binding to Monomer Nucleosomes. Although, as shown in Figure 4, some modulations in cutting frequencies can be observed throughout the curved DNA region of the protein-bound UL4 dinucleosomes, the most distinctive HMG-I(Y)-induced changes in DNase-I cleavage patterns are observed in the T7 promoter region. As noted earlier, the translational position of the second reconstituted nucleosome in the region of the T7 promoter of reconstitutes is variable between different structured DNA fragments (Figure 1). Furthermore, it is known that in the absence of ancillary stabilizing proteins, nucleosomes positioned on long DNA fragments *in vitro* have an inherent tendency to move under low-salt conditions and transiently occupy multiple minor positions centered around a dominant major site (Meersseman et al., 1991; Pennings et al., 1991, 1994; Ura et al., 1995). It is thus conceivable that the marked cleavage changes in the T7 promoter regions observed in the protein-bound curved DNA dimers could be a consequence of HMG-I(Y) kinetically "trapping" and stabilizing T7 promoter DNA in-between two adjacent mobile nucleosomes rather than resulting from actual protein-induced structural changes in the T7 DNA located on the surface of a nucleosome particle. To directly assess this possibility we limited the size of the DNA fragment on which histone cores are assembled in order to drastically restrict, or essentially halt, any *in vitro* mobility (Hayes et al., 1990, 1991a,b). For these experiments, we prepared 142 bp long *Hind*III−*Dde*I fragments covering the region of the T7 promoter and the structured DNAs from each of the different plasmids (Figure 1) and reconstituted these approximately monomer nucleosome-length DNAs into core particles as assessed by micrococcal nuclease digestion, velocity sedimentation, and electrophoretic mobility analyses (data not shown).

Figures 6 and 7 show DNase-I and hydroxyl radical cleavage patterns of reconstituted curved UL1 and rigid BA38 mononucleosomes (containing 142 bp of DNA) with, and without, bound HMG-I(Y). The most striking observation from these figures is that the HMG-I(Y)-induced changes in cleavage patterns seen on these monomer nucleosomes are very similar to those previously observed over the corresponding regions of DNA in HMG-I(Y)-bound dinucleosomes (compare these figures with the results for curved UL4 and rigid BA38 DNAs in Figure 4). For example, as shown in Figure 6, both DNase-I and hydroxyl radical cleavage of HMG-I(Y)-bound UL1 monomer nucleosomes reveal markedly altered DNA cleavage patterns in the vicinity of the T7 promoter. We therefore conclude from the results of these mononucleosome studies and from the cumulative data that the changes in DNA cleavage patterns observed in

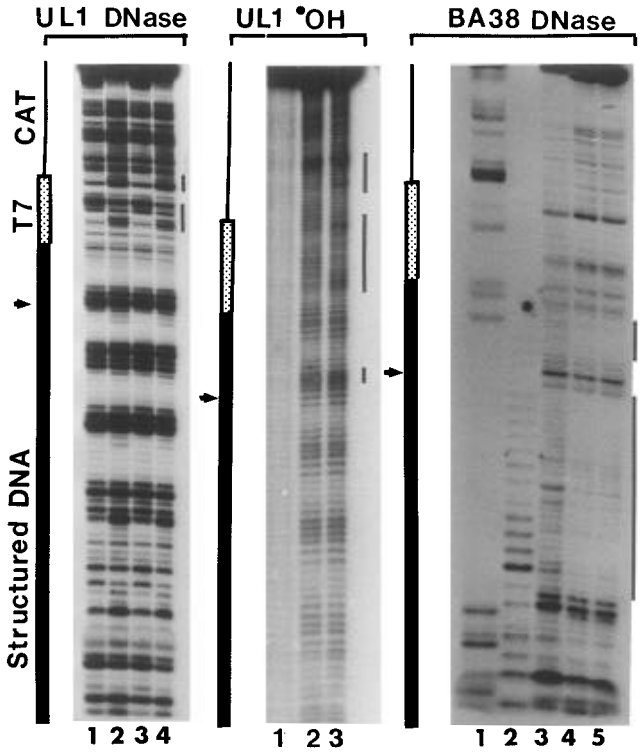

FIGURE 6: DNase-I and hydroxyl radical cleavage of UL1 and BA38 monomer nucleosomes without, and with, bound HMG-I(Y). Panel UL1 DNase-I: Lane 1, mononucleosome without HMG-I(Y); lane 2, mononucleosome plus HMG-I(Y) at a protein:DNA molar ratio of 4:1; lane 3, mononucleosome plus calf thymus HMG-17 at a protein:DNA molar ratio of 4:1; lane 4, mononucleosome plus HMG-I(Y) and HMG-17, both at protein:DNA molar ratios of 4:1. Panel UL1 •OH: Hydroxyl radical cleavage analysis of reconstituted UL1 monomer nucleosomes. Lane 1, free DNA; lane 2, monomer nucleosome without HMG-I(Y); lane 3, mononucleosome plus HMG-I(Y) at a protein:DNA molar ratio of 4:1. Panel BA38 DNase: Lane 1, Maxam−Gilbert G-reaction; lane 2, free DNA; lane 3, reconsituted monomer nucleosome without HMG-I(Y); lanes 4 and 5, reconstituted monomer nucleosomes plus HMG-I(Y) at a protein:DNA molar ratio of 6:1. Other legends as in Figure 1.

HMG-I(Y)-bound UL1 and UL4 chromatin particles are unlikely to result from *in vitro* nucleosome sliding and suggest instead that the alterations are most probably the result of HMG-I(Y)-induced changes in DNA structure on the surface of nucleosome core particles (see Figures 8 and 9 and Discussion).

The other significant DNA cleavage pattern changes observed in HMG-I(Y)-bound chromatin particles were a distinct protection or footprinting of the protein over much of the rigid oligo(dA)·oligo(dT) segment of the reconstituted BA38 dinucleosomes (Figure 4). In agreement with this finding, both DNase-I digestion (Figure 6) and hydroxyl radical cleavage (Figure 7B) of rigid BA38 monomer nucleosomes also show that HMG-I(Y) footprints to a similar region in the oligo(dA)·oligo(dT) tract on the lateral and front surface of these core particles as it does on reconstituted dinucleosomes (Figure 4). As with the BA38 dinucleosomes, it is important to point out that both the DNase-I (Figure 6) and hydroxyl radical (Figure 7B) cleavage patterns of the DNA flanking either side of the HMG-I(Y)-protected region on these monomer nucleosome particles are quite similar in the unbound and the protein-bound particles. We therefore posit that the protection patterns observed on the BA38 monomer nucleosomes result from HMG-I(Y) protein bind-

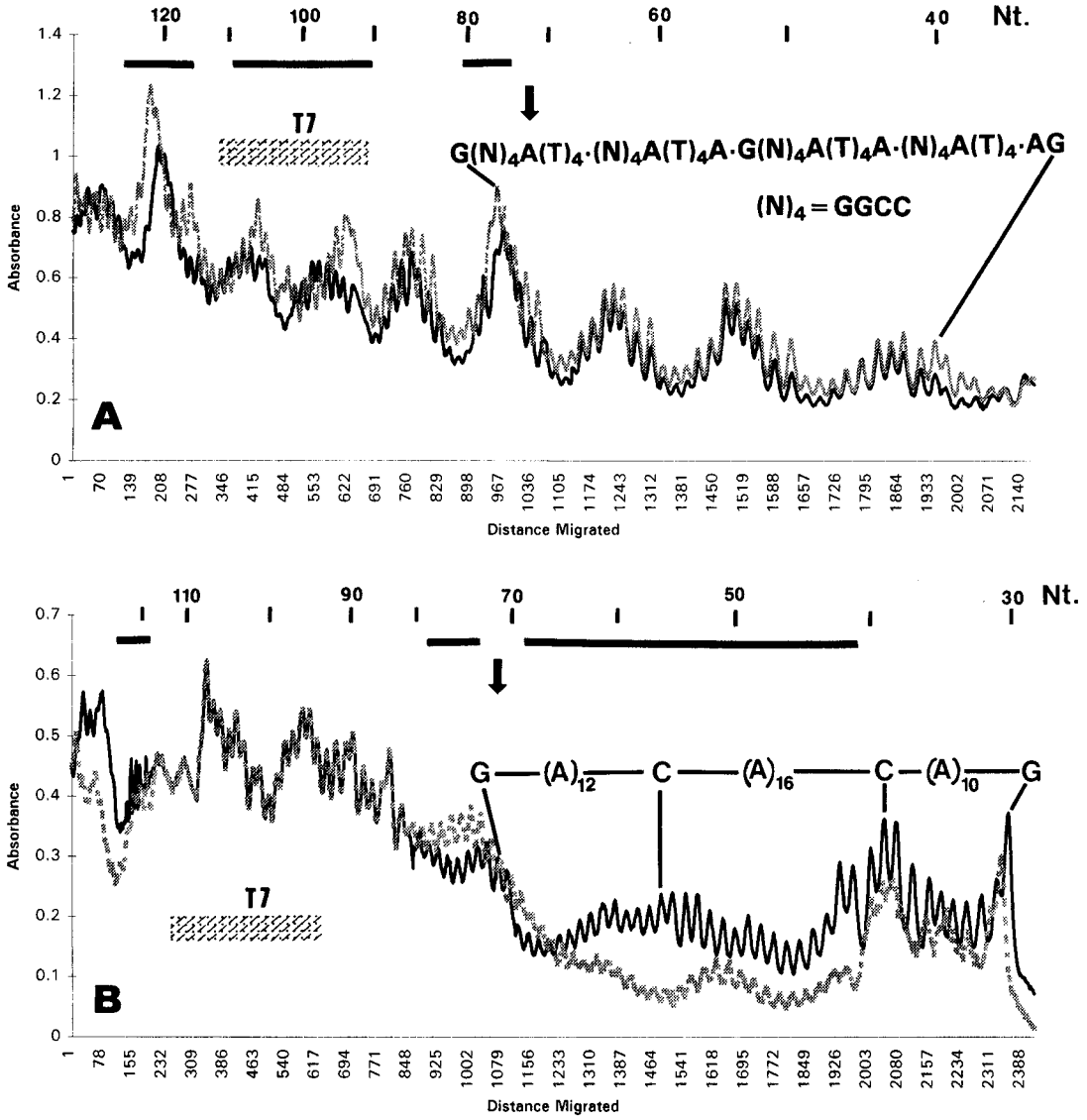

FIGURE 7: Densitometry scans of hydroxyl radical cleavage patterns of HMG-I(Y) on UL1 (panel A) and BA38 (panel B) monomer nucleosomes. Laser densitometry scan of monomer nucleosomes without (solid line) or with bound HMG-I(Y) (dashed lined) at a molar ratio of 4:1, protein:DNA. Distance in nucleotides (Nt) from the 5′ end-labeled *Hind*III site is show across the top of the figure. The position of the T7 promoter (stippled box) and the putative position of the dyad axis of each nucleosome (arrows) are indicated. The horizontal solid lines indicate areas of the gel showing significant changes as a result of HMG-I(Y) binding. The nucleotide sequence of the regions of structured DNA is indicated by the panel inserts.

ing directly to the DNA on the surface of core particles rather than merely being the product of nucleosome sliding.

HMG-I(Y)-Induced Changes in Localized DNA Helical Periodicity on Monomer Nucleosomes. A comparison of the densitometry scans shown in Figure 7A of the hydroxyl radical cleavage patterns of the UL1 monomer nucleosomes with, and without, bound HMG-I(Y) shows what appears to be an alteration in the linear positioning of the major peaks of cutting in, and around, the T7 promoter sequence. That these HMG-I(Y)-induced modulations in cleavage patterns likely represent a localized alteration in the rotational setting of the DNA on the surface of the nucleosome core particle is suggested by the detection of an apparent change in the helical periodicity in the DNA in the region of the T7 promoter when the hydroxyl radical cleavage patterns of Figure 7A are curve-resolved, smoothed, and assessed by Fourier transformation analyses (Figure 8). Detailed analyses (see Materials and Methods) of the variations in hydroxyl radical cleavage frequencies (as exemplified by changes in the absorbance intensity of cleavage bands) of the DNA in UL1 mononucleosomes without bound HMG-I(Y) (solid curve; Figure 8A) gave the expected pattern of alterations of helical periodicity (i.e., number of base pairs per turn) in different regions of the core particle: namely, an underwinding of about 1−1.5 turns of DNA on either side of the dyad axis (arrow) to about 10.7 bp/turn and an overwinding to about 10.0 bp/turn of the reminder of the nucleosomal DNA (Gale & Smerdon, 1988; Hayes et al., 1990). In contrast, the HMG-I(Y)-bound core UL1 particles (dashed line; Figure 8A) exhibited a markedly different overall cleavage pattern: while the helical periodicity of the DNA is quite similar to that of the unbound nucleosome from the region of structured DNA up to the T7 promoter (Figure 8A), from this point on in the core particle the helical periodicity is noticeably altered from that of the DNA in the unbound nucleosome. To better visualize this difference, the resolved curves of the T7 promoter and upstream flanking region shown in Figure 8A were base-line-adjusted and replotted against the nucleotide number of the DNA in the unbound nucleosomes and the results shown in Figure 8B.

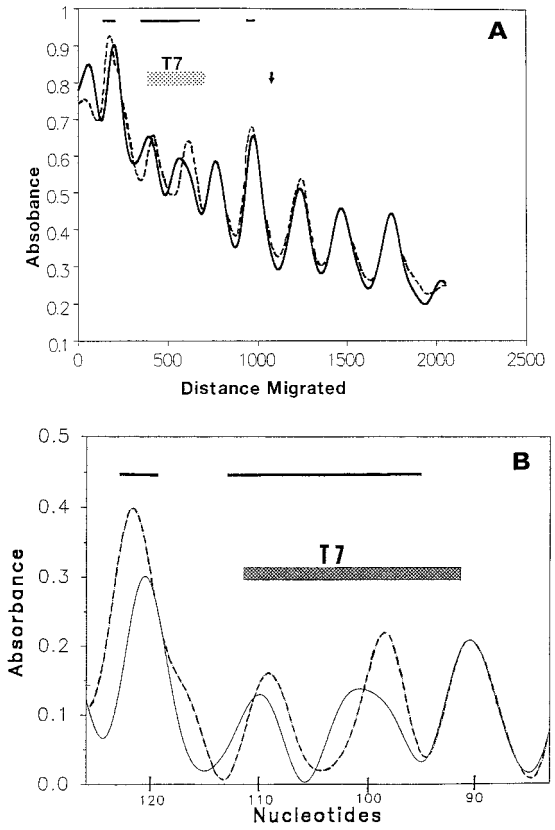

FIGURE 8: (A) Curve-resolved and Fourier-transformed hydroxyl radical cleavage patterns shown in Figure 7A of the UL1 monomer nucleosomes with, and without, bound HMG-I(Y). (B) Base-line adjusted cleavage frequencies in the region of the T7 promoter shown in panel A. Lines and legends as in Figure 7.

As can be clearly seen in this figure, the helical periodicity of the DNA of unbound nucleosomes (solid line) in this region is markedly different from that of the DNA on the HMG-I(Y)-bound core particle (dashed line). Quantitative estimates of these differences were obtained by comparing the relative frequency of hydroxyl radical cutting in this area (Figure 9) of unbound (lower gray bars) and HMG-I(Y)-bound (upper black bars) UL1 DNAs. As expected, the helical periodicity of the unbound DNA in this region of the monomer nucleosome is ~10 bp/turn whereas the apparent periodicity of the HMG-I(Y)-bound DNA varies between about 7 and 13 bp/turn, depending on the region of the core particle analyzed (Figure 9). We interpret these perturbations in helical periodicity as likely reflecting an HMG-I(Y)-induced change in the localized rotational setting of the DNA in this region of the reconstituted UL1 monomer core particles. A similar HMG-I(Y)-induced localized perturbation of helical periodicity and/or rotational setting was also observed for the DNA in reconstituted UL4 monomer nucleosomes (data not shown).

Not all HMG proteins induce these apparent alternations in DNA helical periodicity/rotational setting when bound to nucleosomes. As a control for the above experiments we also bound purified HMG-17 protein to UL1 monomers (Figure 6; UL1 DNase, lane 3; unpublished observations) and did not observe any protein-induced cleavage pattern changes in the region of the T7 promoter. Neither did we see any additive effects on the UL1 monomer cleavage patterns when both HMG-I(Y) and HMG-17 were simultaneous bound to the particles (Figure 6; UL1-DNase, lane

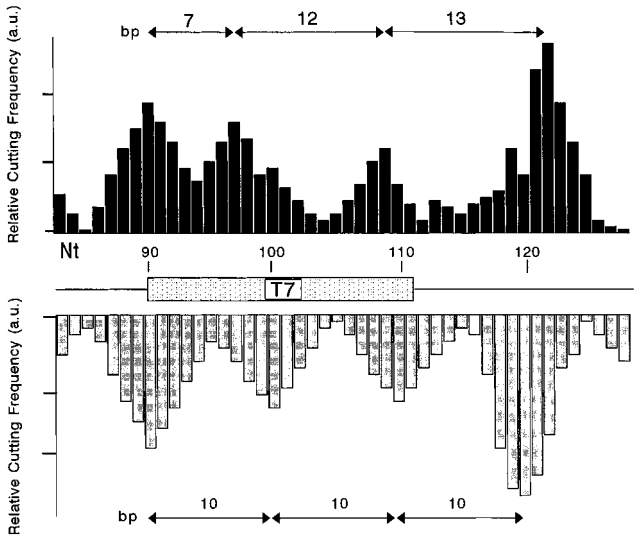

FIGURE 9: Graph of the relative hydroxyl radical cutting frequency (arbitrary units, a.u.) of the T7 promoter region of reconstituted UL1 monomer nucleosomes (from Figure 8B) without (lower gray bars), and with (upper black bars), bound HMG-I(Y). The approximate helical periodicities (in base pairs, bp) of the DNA on different regions of the nucleosome surface are indicated above and below the bar graphs. Other lines and legends as in Figure 7.

4). Likewise, neither histone H1, HMG-1, HMG-2 or protamine were able to induce observable changes in the helical periodicity of the UL1 and UL4 DNAs that had been reconstituted into monomer nucleosomes (data not show). We conclude from these control experiments that the remarkable ability of HMG-I(Y) to induce apparent localized changes in the helical periodicity and/or rotational setting of intrinsically curved DNAs that have been reconstituted into nucleosome core particles is not shared by most other small, positively charged proteins.

DISCUSSION

The results presented clearly demonstrate that HMG-I(Y) binding is significantly influenced by DNA structure, recognizing certain DNA sites when the substrate is free in solution but altering these sites of binding when the same DNA fragment is deformed by wrapping into nucleosomal structures. Although common sites of protein binding occur on all nucleosomes (e.g., at the entrance/exit of DNA on the core particles; Reeves & Nissen, 1993), depending on the actual sequence and/or structure of particular A·T segments, individual reconstituted nucleosomes differ with respect to both HMG-I(Y) binding site selection and the DNA structural changes observed in the bound chromatin particles. Two examples from the present work are of particular interest. The first is the observation that when curved UL1 and UL4 DNAs are reconstituted into chromatin, HMG-I(Y) binding apparently alters the rotational setting, as reflected by a localized alternation in helical periodicity, of the T7 promoter DNA in both monomer and dinucleosome particles (Figures 4−9). As a caveat, it should be noted that in the present experiments it is not easy to unambiguously distinguish localized HMG-I(Y)-induced alterations in helical periodicity from simple protein-induced protection in certain DNA regions and/or slight perturbations of nucleosomal DNA structure due to protein binding. Nevertheless, precedent exists for rotational setting changes in nucleosomal DNA being induced by the binding of small, nonintercalating

molecules such as distamycin (Low et al., 1986). In this connection, it is of interest that the structure of the DNA-binding domains of the HMG-I(Y) protein bear a striking resemblance to the distamycin molecule (Reeves & Nissen, 1990; Evens et al., 1992, 1995; Geierstanger et al., 1994) and *in vitro* these two molecules compete with each other for the same binding sites on DNA (Reeves & Nissen, 1990; Radic et al., 1992).

The second important observation is that in certain cases, such as on the nucleosomes formed from BA38 DNA with its relatively rigid oligo(dA)·oligo(dT) tract, HMG-I(Y) is able to bind to long stretches of A·T-DNA located on the nucleosome surface (Figures 4, 6, and 7B). A potential explanation for this surface binding of HMG-I(Y) to BA38 nucleosomes is that although rigid oligo(dA)·oligo(dT) tracts can be distorted around histone octamers to form nucleosomes, such distortion is energetically unfavorable (Hayes et al., 1991b). The present findings thus suggest that these less stable nucleosomes, while not dissociating in any detectable way, allow for HMG-I(Y) binding in regions of the core particle that are not permissible on nucleosomes with more tightly bound DNAs.

An obvious concern in all of these experiments is whether the changes in cleavage patterns observed upon HMG-I(Y) binding, particularly those seen on the BA38 monomer and dinucleosomes, might be artifactual and perhaps due to the presence of either free DNA, dissociated nucleosomes, or degraded nucleosomes in the preparations. Another concern is whether the observed HMG-I(Y) effects are simply nonspecific ones that might result from the interaction of a variety of small, positively charged, molecules with nucleosomes. We deem all of these possible alternative explanations unlikely for several reasons. First, as noted above, all of these HMG-I(Y)-containing nucleosomes (monomers and dimers) were electrophoretically separated on native nucleoprotein gels from any unbound or degraded nucleosomes, as well as from any free DNA, *after* treatment with cleavage reagents and before subsequent analysis, thus arguing strongly against any spurious mixed contaminations in the samples. Second, the integrity and stoichiometry of the histones in reconstitutes were assessed by SDS−PAGE analysis before and after HMG-I(Y) addition, and no significant degradations or changes in histone composition were observed. Third, the cleavage patterns flanking on either side of the sites of the observed HMG-I(Y)-induced perturbations, particularly in the BA38 nucleosomes, are extremely similar in both the unbound and protein-bound chromatin particles, arguing for a uniformity of the chromatin particles in the experimental populations. And, fourth, the observed effects of HMG-I(Y) are specific in that other small, positively charged proteins (e.g., HMGs 1, 2, and 17 and histone H1) do not, under the same conditions, lead to the same types of structural changes when they bind to nucleosomal DNAs (Figure 6 and unpublished data).

The apparent ability of HMG-I(Y) not only to alter the helical periodicity and/or rotational setting of DNA on the surface of nucleosomes but also to bind certain types of A·T stretches located on the surface of core particles has numerous implications for how this non-histone protein might function *in vivo* as an "architectural" transcription factor (Grosschedl et al., 1994; Bustin & Reeves, 1996). The HMG-I(Y) proteins are known to specifically interact with a number of transcription factors both *in vitro* and *in vivo* including NF-κB and ATF-2 (Thanos et al., 1994), Elf-1 (John et al., 1995), and Oct/POU proteins (Abdulkadir et al., 1995; Leger et al., 1995). Thus, one of the more interesting and plausible *in vivo* functions for HMG-I(Y) might be for "targeting" of sequence-specific transcription factors to their DNA recognition sites that have been occluded by inhibitory nucleosomes and subsequently leading either to the local rotational repositioning of the DNA on these nucleosomes and/or to participation in their disruption/displacement, thereby facilitating transcription factor binding and gene activation. From these and other data (Reeves & Nissen, 1993), it is apparent that HMG-I(Y) binding, by itself, does not induce nucleosome disruption/displacement. Nevertheless, given the observations reported here, it is reasonable to suspect that the HMG-I(Y) protein might act in conjunction with other factors, such as those in the multiprotein SWI/SNF "general" activator complex [reviewed in Wolffe (1994a)], to facilitate remodeling of localized regions of chromatin *in vivo*. We are currently investigating these possibilities.

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