The Non-histone Chromatin Protein HMG1 Protects Linker DNA on the Side Opposite to That Protected by Linker Histones

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Linker histones and HMG1/2 constitute the two major proteins that bind to linker DNA in chromatin. While the location of linker histones on the nucleosome has attracted considerable research effort, only a few studies have addressed the location of HMG1 in the particles. In this study, we use a procedure based on micrococcal nuclease digestion of reconstituted nucleosomal particles to which HMG1 has been bound, followed by analysis of the protected DNA by restriction nuclease digestion, to locate the HMG1 binding site. Nucleosomal particles were reconstituted on a 235-base pair DNA fragment, which is known to be a strong nucleosome positioning sequence. The results unequivocally show that HMG1 protects linker DNA on one side of the core particle. Importantly, and possibly of physiological relevance, the linker DNA site protected by HMG1 was located on the side opposite to that already shown to be protected by linker histone binding.

EXPERIMENTAL PROCEDURES

Preparation and Purification of HMG1 and DNA—HMG1 was purified from chicken erythrocytes by the procedure of Adachi et al. (14). The cloning and purification of the 235-bp fragment from pgUB (13) was described recently (8).

Reconstitution of Core Nucleosomes and HMG1 Binding—Reconstitutions of core nucleosomes were carried out by salt dialysis (15) at 4 °C. Core histone octamers (16) were mixed with 60 μg of DNA in 0.6 ml of 2.2 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.25 mM EDTA. HMG1 was mixed with reconstituted core particles prepared with results concerning linker histone protection on particles reconstituted on the same sequence (8).

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Micrococcal Nuclease Digestions of Reconstituted Core Nucleosomes and HMG1-containing Core Nucleosomes—The solution containing reconstituted nucleosomes was made 1 mM with respect to CaCl2, 1 unit of MNase ( Worthington) was added per 5 μg of DNA and incubated for up to 8 min. For further details of the digestion protocol, see Ref. 8. The
The nucleosomal DNA present in the reconstituted particle preparations will not be detectable by sequencing gels, but it showed a pattern totally unrelated to the pattern produced by the 146-bp core particle fragment (not shown). For example, neither of the core particle boundaries could be seen; thus this fragment must arise from cleavages elsewhere on the 235-bp fragment and was not considered as resulting from further protection imposed on the core particle by bound HMG1.

naked DNA fragment was digested with 0.2 unit of MNase/5 μg of DNA for 5 min.

Gel Purification of DNA, End Labeling, and Restriction Nuclease Digestion—These were performed exactly as described earlier (8).

Cross-linking of Histone Octamer to DNA in Reconstituted Core Nucleosomes—Cross-linking was carried out with 0.1% glutaraldehyde as described in Ref. 8.

RESULTS AND DISCUSSION

To study the protection against MNase cleavage on nucleosomal DNA provided by HMG1 binding, we used the protocol introduced by Dong et al. (17) and later applied by other investigators in the field. Briefly, the reconstituted nucleosomal particles were digested with MNase to cleave off portions of linker DNA that were not protected by interaction with proteins. The proteins were then removed by proteinase K digestion, the protected DNA fragments of desirable lengths were purified from polyacrylamide electrophoretic gels, end-labeled, and further subjected to restriction nuclease digestion. The lengths of the resulting restriction fragments were determined in sequencing gels and used to deduce the location of the histone octamer on the sequence and the protection of linker DNA by HMG1 (for a schematic, see Fig. 3C).

The success of reconstitution was followed by gel retardation assays of the kind shown in Fig. 1. Addition of the histone octamer to the 235-bp pGUB fragment led to the appearance of a retarded band, reflecting the formation of a core nucleosome on the DNA fragment (the core nucleosome differs from a core particle by the presence of “linker” DNA on both its sides). Further addition of HMG1 to the core nucleosome led to the formation of a particle, which was further retarded on the gel.

MNase digestion of this mixture (lane 4, Fig. 1), the core nucleosome/DNA mixture (lane 2), and of naked DNA as a control, reproducibly produced DNA patterns such as those shown in Fig. 2, A, B, and C, respectively.

Digestion of the core nucleosome preparation revealed the expected kinetic pause of digestion at 146 bp (Fig. 2A), which results from DNA protection by the histone octamer. The DNA patterns resulting from digestion of the HMG1/core nucleosome reconstitute contained, in addition to the core particle DNA band, an additional band of lower stability at ~168–170 bp. Finally, digestion of naked DNA, even under milder conditions, did not produce any DNA bands of 146 and 170 bp length (Fig. 2C). It must be emphasized that the small amount of free DNA present in the reconstituted particle preparations will not in any event contribute to the patterns observed with these particles, since under the conditions of digestion used to obtain...
DNA from these particles naked DNA is completely digested to small fragments unobservable on gels.

The 146-bp DNA bands from both the core nucleosome and the HMG1/core nucleosome MNase digests, as well as the ∼170-bp band from the HMG1/core particle preparation, were further subjected to XhoI restriction nuclease digestion, and the lengths of the resulting fragments were determined by sequencing gels (Fig. 3A). In accordance with published data (13) and our previous results (8), the GUB sequence placed the histone octamer in one major translational position, defined by DNA fragments 108 and 39 bases in length. Moreover, the same major core particle position was present in the core nucleosome (not shown) and in the HMG1/core nucleosome reconstitute (Fig. 3A, lane 2).

The pattern of XhoI cleavage of the ∼170-bp band in the MNase digestion pattern of the HMG1/core nucleosome reconstitute revealed two major bands, 132 and 39 bases in length. Since the 3’ border of the protein-protected region was the same as in the core particle, and the 5’ border was shifted 24 bp in the 5’ direction, HMG1 must protect linker DNA on only one side of the core particle (see Fig. 3C). This result is at variance with the result obtained with the X. borealis 5 S rDNA (10, 11); the latter result may be due to the artifacts connected to the use of the 5 S gene sequence for MNase-based assays of protein protection. We have been able to observe the cleavages corresponding to 5/15 bp protection reported previously (10, 11) upon digestion of free DNA or core particles (12).

To interpret such data unambiguously, we must eliminate the possibility that HMG1 causes a short range shift of the position of the histone octamer along the DNA fragment. If such a shift were to take place, then the 132/29 pattern obtained upon XhoI cleavage could reflect a 24-bp shift of the core in the 5’ direction and protection of the same length of DNA of (now linker) DNA at the 3’ end by HMG1. To exclude such a possibility, the analysis was repeated after cross-linking the protein to the DNA following the reconstitution of the core nucleosome, but before addition of HMG1. The results obtained on the cross-linked material were indistinguishable from those presented above (compare Fig. 3A with Fig. 3B). This observation reinforces the interpretation presented in Fig. 3C.

The results of these experiments indicate that HMG1 bound to the core nucleosome protects linker DNA on only one side of the core particle. Interestingly, and potentially of physiological relevance, linker histone protects linker DNA on the opposite side of the core particle reconstituted on the same sequence (8). Although our data provide no information on in vivo binding, this issue may be directly related to the possibility of the two proteins competing for binding at the nucleosome, with all ensuing structural and function consequences of such a competition (for a detailed discussion, see Ref. 1). Indeed, early evidence that chromatosome-sized units containing either H1 or HMG1 could be isolated from MNase digests of whole chromatin (18, 19) would argue indirectly that the binding of the two classes of linker proteins might be mutually exclusive, and the two proteins might compete with each other for binding to the nucleosome. That the two proteins compete for binding to four-way junction DNA (20, 21) or cisplatin-modified DNA (22) has been demonstrated recently. Preliminary experiments in our laboratory show that this may also be the case for reconstituted nucleosomes. It should be emphasized that even though the two protected regions are on “opposite ends” of the nucleosomal DNA, those ends are probably closely juxtaposed in the nucleosome, and thus competition or mutual exclusion is still possible. The resolution of this important issue and its relation to transcriptional regulation will require in situ studies addressing the question of whether the transition between transcriptionally active and inactive state of specific gene regions is accompanied by mutual replacement of the two protein classes.

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