High affinity binding of proteins HMG1 and HMG2 to semicatenated DNA loops

ABSTRACT

Of all DNA structures described so far with which HMG1 and HMG2 interact, we have found that Form X, a DNA loop with a semicatenated DNA junction at its base, is the structure with the highest affinity by more than 4 orders of magnitude. This suggests that, if similar structures exist in the cell nucleus, one of the functions of these proteins might be linked to the remarkable property of DNA hemicatenanes to associate two distant regions of the genome in a stable but reversible manner.

INTRODUCTION

Background Proteins HMG1 and HMG2, two of the most abundant non histone proteins, have been known for more than 25 years (for a review see), and their function has been the subject of varied investigations, especially since it was found that they contain a domain of homology with many proteins implicated in the control of development or of differentiation. As examples of recent studies are their immunocytochemical localization, the deletion of the gene coding for HMG1 by homologous recombination in transgenic mice, the effect of HMG1 or of the HMG domain on the assembly of certain nucleoproteic complexes, the observation of the binding of HMG1 to Oct and Hox proteins, to nuclear hormone receptors, or to p53, the influence of HMG1 on the circularization of short DNA fragments, on V(D)J rearrangement in vitro, or on transcription. HMG1 has also been recently implicated as a mediator of endotoxin lethality. An important way of studying the function and the mechanism of action of proteins HMG1/2 has obviously been the search for molecular partners, and, starting from the assumption that these chromatin proteins probably interact with DNA, many studies have been performed to study the sequences or the DNA conformations with which the proteins interact preferentially. For some HMG-domain proteins, specific binding sequences have been identified, but no such sequences have been found for proteins HMG1 and HMG2 themselves, which interact only weakly with double-stranded DNA. However, it has been shown that HMG1/2 could form complexes with several kinds of non-classical DNA structures: supercoiled circles, platinated DNA, UV-modified DNA, bulge loops, and four-way junctions. As the interactions with four-way junctions were stronger than others, they have been the object of particular studies. Thus, the image of HMG1 that has prevailed is of "an all-purpose DNA-bending, -wrapping, and -looping factor that can be recruited for transcription, DNA repair, and recombination". In the course of our studies with CA microsatellites, we have observed that a protein present in nuclear extracts of cultured monkey cells formed specific retarded complexes with a DNA fragment containing a tract of the poly(CA)· poly(TG) sequence (Fig. 1A). The purification of the DNA-binding activity yielded two proteins which were identified as HMG1 and HMG2 (Fig. 1B). And the DNA contained in the complexes was present as a new form ("Form X",), with a mobility lower than the regular double-stranded fragment, which was bound by purified HMG1 and HMG2 (Fig. 1C) and which has been identified as the semicatenated DNA loop which is schematically represented on the Figure. This structure consists in a double-stranded DNA loop at the base of which DNA duplexes cross and form a knot in which one of the strands of one duplex passes between the two strands of the other duplex, and reciprocally. Here we have studied the interactions of HMG1/2 with Form X, and found that these proteins bind much more strongly to semicatenated

DNA junctions than to any other known DNA substrate.

CONCLUSION

Conclusions Of all DNA structures described so far with which HMG1 and HMG2 interact, we have found that Form X, a DNA loop with a semicatenated DNA junction at its base, is the structure with the highest affinity by more than 4 orders of magnitude, suggesting that the possibility should be studied that such DNA hemicatenanes might exist in the cell nucleus. In addition, a role for the HMG domain in the formation or the stabilization of higher order chromatin structures has often been suggested (reviews in), and our results go further in the same direction, by suggesting that one of the functions of HMG1/2 might be linked to the remarkable property of Form X to associate two distant regions of the genome in a stable but reversible manner.