Vol. 143, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1987 Pages 260-265

SPECIFIC A·T DNA SEQUENCE BINDING OF RP-HPLC PURIFIED HMG-I

Terry S. Elton, Mark S. Nissen, and Raymond Reeves

Programs in Biochemistry/Biophysics and Genetics, and Cell Biology,
Washington State University, Pullman, Washington 99164-4630

Received January 20, 1987

SUMMARY: HMG-I (α-protein) is a high mobility group protein which r .ognizes and binds specifically to A·T rich double stranded DNA. We have investigated, by electrophoretic shift assays and DNase I footprinting, the ability of reverse-phase high performance liquid chromatography purified HMG-I to bind to specific A·T rich duplex DNA sequences. We show here that when HMG-I is isolated and purified under denaturing conditions it retains its specific A·T DNA binding activity. These results suggest that reverse-phase high performance liquid chromatography to be the method of choice for the preparation of HMG-I.

It is well accepted that sequence-specific DNA binding proteins play important roles in cellular processes such as transcription, replication, repair and recombination. Recently, Solomon et al. (1) have shown that the high mobility group protein, HMG-I (α-protein), is an A·T sequence-specific duplex DNA binding protein. Although the function of HMG-I is not known, recent reports have suggested that A·T rich DNA sequences may be sites of attachment to the nuclear scaffold or matrix (2-4). Furthermore, we have shown by polycolonal antibodies that HMG-I is present in the nuclear matrix (manuscript submitted). Thus, HMG-I may play an important role in nuclear matrix-DNA interactions in vivo.

Previous studies from this laboratory have shown that ion-pair reverse-phase high performance liquid chromatography (RP-HPLC) is a quick and convenient method for obtaining essentially pure preparations of HMG-I (5,6). For some proteins the utility of a RP-HPLC purification procedure is questionable since the secondary and tertiary structures are not preserved during chroma-

Abbreviations: RP-HPLC, reverse-phase high performance liquid chromatography; HMG, High Mobility Group protein.

0006-291X/87 $1.50

tography carried out under denaturing conditions (7-10). However, we demonstrate here that RP-HPLC purified HMG-I, which has been isolated under denaturing conditions, retains its ability to recognize and bind to specific A·T rich duplex DNA sequences.

MATERIALS AND METHODS

Crude HMG protein samples were obtained by extracting Friend erythroleukemic mouse cells with 5% perchloric acid (11). HMG-I was purified by subjecting the crude HMG protein samples to ion-pair RP-HPLC as previously described (5,6). Following chromatography the purity of HMG-I was determined by acid-urea polyacrylamide gel electrophoresis (12). The protein concentration was determined by the Bradford protein assay (13) using bovine serum albumin as a standard.

HMG-I gel electrophoresis DNA binding shift assays (14) were performed by incubating HMG-I with a ^{32}P end-labeled 295 bp BclI-EcoRI restriction fragment containing the 3' untranslated tail region of the bovine Interleukin-2 (bIL-2) cDNA excised from the plasmid vector pbIL-2 (see Fig. 1A) (15). Binding reactions (25 µl) contained 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA and 5% glycerol. Reactions also contained 2 µg of poly(dI-dC):poly(dI-dC) competitor DNA. After 10-30 min. incubation at room temperature, the resulting protein-DNA complexes were resolved in a low-ionic strength 4% polyacrylamide gel (acrylamide:bisacrylamide weight ratio of 30:1). After electrophoresis the gel was dried and autoradiographed at -70°C with an intensifying screen.

DNase I footprint assays were performed as described previously (16). Briefly, using the single stranded M13 clone MbIL-2, a 5' end-labeled M13 universal primer was hybridized and elongated with Klenow DNA polymerase. This construct was then incubated with RP-HPLC purified HMG-I and digested with DNase I using conditions as described (16). The DNase I footprint reactions were electrophoresed in 6% polyacrylamide gels and autoradiographed. Localization of the HMG-I binding sites was achieved by performing Sanger dideoxy sequencing reactions (17) in parallel with the DNase I footprinting experiments.

RESULTS

Recently, Strauss and Varshavsky (14) have purified HMG-I (α-protein) using a combination of three nondenaturing chromatographic steps. Varshavsky's laboratory has also shown that nondenatured HMG-I (α-protein) can recognize any stretch of six A·T base pairs in duplex DNA. We have used electrophoretic shift assays (14) and DNase I footprinting (16) to investigate the DNA binding activity of RP-HPLC purified HMG-I prepared under denaturing conditions in less than 25 min. (5,6).

Figure 1A shows the restriction map of a full-length bovine Interleukin-2 (bIL-2) cDNA clone isolated as a 791 bp EcoRI restriction fragment from the plasmid vector pbIL-2 (15). This fragment can be further subdivided into a 496 bp EcoRI-BclI fragment and a 295 bp BclI-EcoRI fragment. The 295 bp

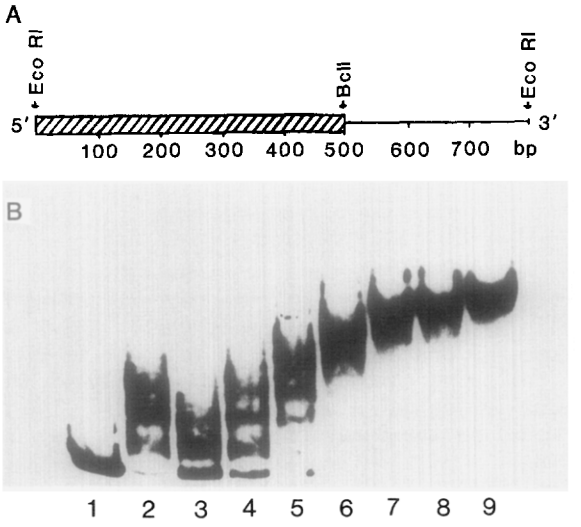

Figure 1. A. Restriction enzyme map of the 791 bp cDNA clone of the bIL-2 gene, showing the open reading frame for the protein coding region (hatched region) and the nontranslated tail region. B. Electrophoretic mobility shift assay of a 295 bp BclI-EcoRI restriction fragment. End-labeled 295 bp 3' untranslated tail (0.5 ng of DNA, 10,000 cpm per binding reaction) was incubated with a fixed amount of competitor DNA (2 µg of poly(dI-dC): poly(dI-dC) per binding reaction) and with increasing amounts of RP-HPLC purified HMG-I before electrophoresis in a low ionic strength 4% polyacrylamide gel. Lane 1, free DNA fragment. Lane 2, 10 ng of HMG-I in the absence of competitor DNA. Lanes 3-9, contain 10, 20, 30, 40, 50, 60 and 70 ng of HMG-I respectively.

restriction fragment, which contains the entire bovine IL-2 3' untranslated region, is abundant in A·T sequences. Therefore, this fragment is ideal for investigating HMG-I DNA binding activity.

Figure 1B shows the results of an HMG-I gel electrophoresis DNA binding shift assay. RP-HPLC purified HMG-I was incubated with the end-labeled 295 bp BclI-EcoRI fragment in the presence of 2 µg poly(dI-dC):poly(dI-dC) competitor DNA. Protein-DNA complexes were then separated from free DNA by electrophoresis through a low ionic strength 4% polyacrylamide gel (14) and visualized by autoradiography. Lane 1, shows the migration of free DNA fragment. Lanes 2-9 show that when RP-HPLC purified HMG-I is incubated with the labeled DNA fragment specific protein-DNA complexes are formed. The reaction mixture shown in Lane 2 did not contain competitor DNA. These results suggest that multiple HMG-I binding sites are present on the 295 bp fragment. Similar experiments were also performed on end-labeled 496 bp

EcoRI-BclI fragment. Although this DNA fragment contains several A·T rich sequences (15), HMG-I does not seem to form any specific protein-DNA complexes (data not shown). Thus HMG-I seems to have a binding preference to certain A·T rich DNA sequences such as the 3' untranslated region, but not the coding region, of bIL-2.

To localize the HMG-I binding sites on the bIL-2 cDNA, DNase I footprinting experiments were performed. As described in "Methods" a double stranded MbIL-2 construct was partially digested with DNase I in the presence or absence of RP-HPLC purified HMG-I. Figure 2, lanes 1 and 6 show the DNase I digestion pattern in the absence of HMG-I. Lane 2-5 show the DNase I digestion pattern in the presence of increasing amounts of HMG-I. It is obvious from these results that HMG-I protects at least 4 regions, labeled A, B, C and D. By performing Sanger dideoxy sequencing reactions (17) in parallel with the DNase I footprinting experiments we were able to determine the DNA sequences to which HMG-I binds in regions A, B, C and D (Fig. 2). The results show that regions A, B, C and D are all A·T rich DNA sequences. If the DNase I/polyacrylamide gels were electrophoresed longer at least five more HMG-I binding sites were detected in the 3' untranslated region of bIL-2 cDNA (data not shown). Under the same conditions the 5' coding region of the bIL-2 cDNA did not show specific binding of HMG-I although this region of the cDNA contains several stretches of A·T rich sequence which might have been suspected, before hand, to bind this protein (1).

DISCUSSION

A previous study has shown that HMG-I isolated under nondenaturing conditions can bind to specific A·T rich sequences (1). However, the purification procedure developed by Strauss and Varshavsky (14) is very time consuming in that it involves the use of three separate chromatographic columns (phosphocellulose, DNA-Sephacryl, and hydroxyapatite). We have previously shown that HMG-I can be purified from crude nuclear extracts in less than 25 min. under denaturing conditions by RP-HPLC.

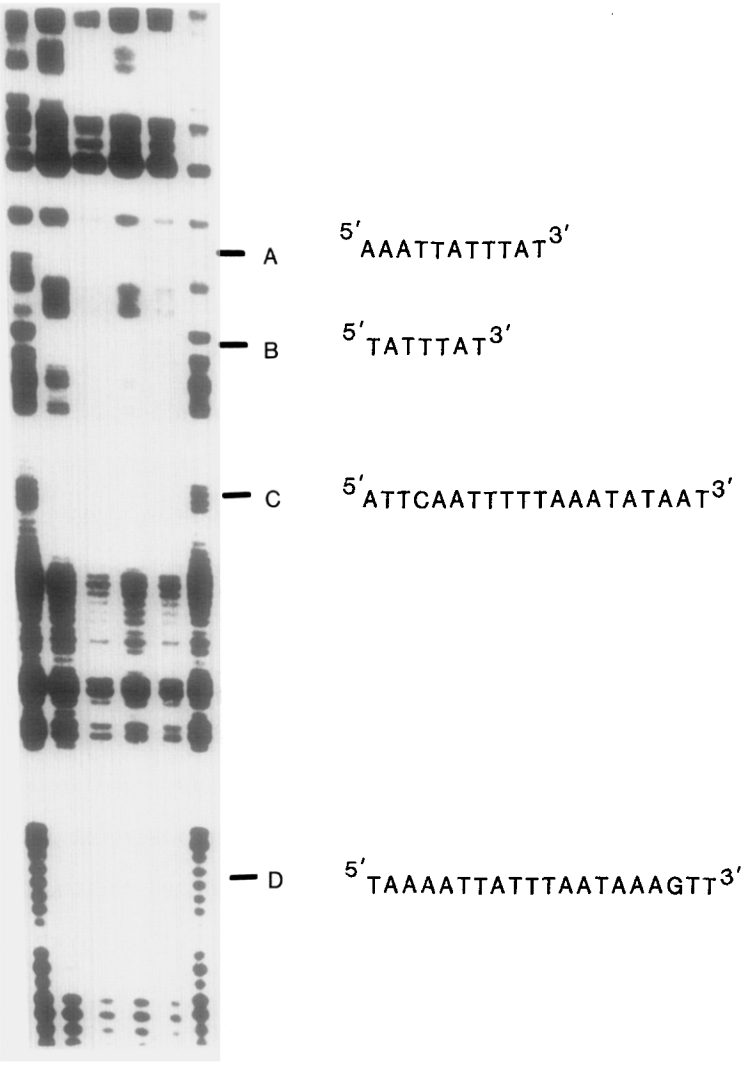

Figure 2. DNase I footprint analyses of RP-HPLC purified HMG-I binding sites in the 3' untranslated tail region of bIL-2 cDNA. Lanes 1 and 6 show control DNase I digestion patterns in the absence of HMG-I. Lanes 2-5 show the DNase digestion patterns in the presence of increasing amounts of HMG-I (25, 50, 75 and 100 ng). Four protected sites within the 3' untranslated tail region of bIL-2 are denoted A, B, C and D. The DNA sequences protected by HMG-I (shown at the right) were deduced by Sanger dideoxy sequencing.

The results presented here demonstrate that RP-HPLC purified HMG-I can form specific protein-DNA complexes. Moreover we show that RP-HPLC purified HMG-I binds specifically to A·T rich regions present in the bIL-2 cDNA 3' untranslated tail. The reason why HMG-I does not seem to bind to short A·T rich sequences of DNA in the 5'-coding region of bIL-2 cDNA is not known but

may reflect an influence of flanking DNA sequences in determining the specificity of binding of HMG-I to its preferred sites.

The amino acid composition of HMG-I (6) suggests that it, like HMG-14 and -17, is devoid of secondary and tertiary structure due to the lack of hydrophobic amino acids. In fact, circular dichroism experiments performed in our laboratory have shown that the α-helix content of HMG-I is very low (manuscript in prepartion). Thus these results seem to be consistent with our observations that exposure to denaturing conditions does not seem to effect the DNA binding activity of HMG-I. Since RP-HPLC is a rapid and efficient means of purifying active HMG-I (compared to classical protein purification procedures), we find this technique to be the method of choice for the preparation of HMG-I.

ACKNOWLEDGEMENT

This work was supported by National Science Foundation Grant DCB-8602622.

REFERENCES

1. Solomon, M. J., Strauss, F., and Varshavsky, A. (1986) Proc. Natl. Acad. Sci. USA 83, 1276-1280.
2. Mirkovitch, J., Mirault, M. E., and Laemmli, U. K. (1984) Cell 39, 223-232.
3. Cockerill, P. N., and Garrard, W. T. (1986) Cell 44, 273-282.
4. Gasser, S. M., and Laemmli, U. K. (1986) Cell 46, 521-530.
5. Elton, T. S., and Reeves, R. (1985) Anal. Biochem. 144, 403-413.
6. Elton, T. S., and Reeves, R. (1986) Anal. Biochem. 157, 53-62.
7. Benedek, L., Dong, S., and Karger, B. L. (1984) J. Chromatogr. 317, 227-243.
8. Ingraham, R. H., Lau, S. Y. M., Taneja, A. K. and Hodges, R. S. (1985) J. Chromatogr. 327, 77-92.
9. Lau, Y. M., Taneja, A. K., and Hodges, R., S. (1984) J. Chromatogr. 317, 129-140.
10. Luiken, J., VanDerZee, R. and Welling, G. W. (1984) J. Chromatogr. 284, 482-486.
11. Goodwin, G. H., Walker, J. H., and Johns, E. W. (1978) In: The Cell Nucleus, ed., Busch, H., Vol. 6, pp. 181-219, Academic Press, New York.
12. Panyim, S., and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346.
13. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254.
14. Strauss, F., and Varshavsky, A. (1984) Cell 37, 889-901.
15. Reeves, R., Spies, G., Nissen, M. et al. (1986) Proc. Natl. Acad. Sci. USA 83, 3228-3232.
16. Pruijn, G. J. M., VanDriel, W., and Van Der Vliet, P. (1986) Nature 322, 656-659.
17. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.