Nonhistone chromatin proteins HMG-14 and HMG-17 bind preferentially to single-stranded DNA

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Received 27 May 1981

ABSTRACT

Proteins extracted from chicken erythrocyte chromatin with 0.35 M NaCl were subjected to sequential chromatography on columns containing immobilized double-stranded and single-stranded DNA's. Two-dimensional electrophoresis of protein fractions revealed that HMG-14 and HMG-17 are among the proteins that are retained by the single-stranded DNA column in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5) after having failed to be retained by the double-stranded column under the same conditions. That suggests that those two proteins possess preferential affinity for single-stranded DNA. Further evidence for that was provided by chromatography of purified HMG-14 and of purified HMG-17 on single-stranded and double-stranded DNA columns. We discuss the possible relevance of our results to suggested functions of HMG-14 and HMG-17.

INTRODUCTION

There is considerable evidence that the low molecular weight HMG¹ nonhistone chromatin proteins are preferentially associated with transcribed genes in chromatin. Dixon and coworkers (1) have shown that in trout testis, H6 (an HMG protein) occurs in stoichiometric quantities in isolated mononucleosomes that are enriched in transcribed DNA sequences. Weisbrod et al. (2,3) have demonstrated that HMG-14 and HMG-17 confer DNase-I sensitivity upon transcribed genes in chick red cell chromatin. Those two proteins appear to bind preferentially in vitro to nucleosomal core particles that contain transcribed sequences (3-5). Because of their association with transcribed DNA sequences, it seems likely that the low molecular weight HMG proteins play some role in transcription, but it is not known what that role is. Nor is it known how the low molecular weight HMG proteins recognize nucleosomes containing transcribed genes or how the proteins confer DNase-I sensitivity upon those nucleosomes.

In this paper, we report studies on the interactions of HMG-14 and HMG-17 with purified DNA that may be related to the proteins' interactions with DNA in chromatin. Our initial information is obtained by sequential chromatography of NaCl dissociated nonhistone chromatin proteins on columns containing

immobilized double-stranded DNA and single-stranded DNA. We have previously reported that high molecular weight HMG proteins (HMG-1, HMG-2, and HMG-E) are selectively retained by the single-stranded DNA column when sequential chromatography is carried out at 0.2 M NaCl (6). We noted in that report that HMG-14 and HMG-17 did not appear to share that property. [Nor did any other abundant nonhistone chromatin proteins in 0.35 M NaCl extracts of calf thymus, chicken erythrocyte, or cultured hepatoma cell chromatin (7)]. As we began analyzing protein fractions by acid/urea gel electrophoresis, we realized that the low molecular weight HMG proteins were quite difficult to detect in the gel system that we used in our original analyses. As we show in this paper, when an electrophoresis system is used that allows detection of HMG-14 and HMG-17, it becomes clear that they share with the high molecular weight HMG proteins the property of being selectively retained by a single-stranded DNA column at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). We interpret this to indicate that HMG-14 and HMG-17 possess preferential affinity for single-stranded DNA, at least under those solvent conditions. We have verified that interpretation by chromatography of the purified proteins on DNA columns.

MATERIALS AND METHODS

Preparation of DNA cellulose columns. Salmon DNA (Sigma, Type III) was purified by chloroform/isoamyl alcohol (24:1) extraction and sheared for 1.5 min in a Sorvall Omnimixer at full speed. Double-stranded DNA cellulose was prepared by the method of Alberts and Herrick (8) from the purified salmon DNA and Cellex N1 cellulose (Bio-Rad). Single-stranded DNA regions were removed from the putative double-stranded DNA cellulose column by circulating 100 units of S1 nuclease (Calbiochem) through the column for 24 hr at room temperature in 5% glycerol/10 mM NaCl/1 mM ZnSO_4 /50 mM sodium acetate (pH 4.5). The resulting double-stranded DNA column (3.5 x 16 cm) contained about 15 mg of DNA. Single-stranded DNA cellulose was prepared by covalently linking heat-denatured salmon DNA to Cellex N1 cellulose by the method of Litman (9). The single-stranded DNA column (2.5 x 30 cm) contained about 100 mg of DNA. The amounts of DNA bound to cellulose were determined by the method of Hosoda and Moise (10). The DNA cellulose columns were operated at flow rates of 100-200 ml/hr at 4° under a head of 1.5-2 m.

Preparation of protein samples. Chicken blood was collected at the Campbell Soup Company in Tecumseh, Nebraska. Nuclei were isolated with minor modification (11) of previously published methods (12). Chromatin was prepared

by homogenizing nuclei in 1 mM Tris-Cl (pH 7.5). Chromatin, at a DNA concentration of 2 mg/ml, was brought to 0.35 M NaCl and stirred for 30 min to extract proteins, including the HMG proteins (13). The protein extract was obtained as a supernatant after a 30 min, 25,000 g centrifugation. All steps in obtaining the extract were carried out at 0-4°.

HMG-14b and HMG-17 were purified from the 0.35 M NaCl-extracted proteins by chromatography on phosphocellulose and CM-Sephadex, as previously described (14).

Gel electrophoresis. Acid/urea gel electrophoresis was carried out with the system of Panyim and Chalkley (15) in slabs polymerized from 20% acrylamide and 0.3% bis-acrylamide. Two-dimensional gel electrophoresis was performed as described in detail elsewhere (11) with nonequilibrium pH gradient electrophoresis in the first dimension (16) and polyacrylamide/SDS gel electrophoresis in the second dimension. Samples for electrophoresis were concentrated by precipitation at 25% trichloroacetic acid, washed with acetone, and dissolved in "lysis buffer" of O'Farrell (17) or in 0.9 M acetic acid.

Other procedures. Protein concentrations were determined by the Coomassie Blue binding assay of Bradford (18). Conductivities were determined with an Electro-Mark Analyzer (Markson Science, Inc.).

RESULTS

Sequential DNA chromatography of 0.35 M NaCl-extracted proteins. The 0.35 M NaCl extract of chicken erythrocyte chromatin was diluted to 0.2 M NaCl and subjected to sequential chromatography on double-stranded and single-stranded DNA cellulose columns that had been equilibrated to 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). [Those solvent conditions were chosen on the basis of earlier work (6), in which we showed that the high molecular weight HMG proteins (HMG-1, HMG-2, and HMG-E) all failed to be retained by double-stranded DNA columns in 0.2 M NaCl/1 mM Tris-Cl, pH 7.5.]. The flowthrough from the double-stranded DNA cellulose column was applied directly to the single-stranded DNA cellulose column, and after that column was washed with 0.2 M NaCl/1 mM Tris-Cl (pH 7.5), bound proteins were eluted by applying solutions of higher NaCl concentrations. Figure 1 shows a two-dimensional electrophoretic analysis of the proteins eluted from single-stranded DNA cellulose with 0.5 M NaCl/1 mM Tris-Cl (pH 7.5). As expected from our earlier work (6), HMG-1, HMG-2, and HMG-E are the most prominent members of that group of proteins. The two other most prominent proteins that bind to single-stranded DNA

Figure 1. Two-dimensional gel electrophoresis of chicken erythrocyte chromatin proteins that were retained by a single-stranded DNA column after having failed to be retained by a double-stranded DNA column. Work that allowed identification of the HMG proteins on this electrophoretogram is described in detail elsewhere (11). The identifications are based on TCA solubility properties of the HMG proteins and on electrophoresis of individual HMG proteins purified as described in reference 14.

columns after having failed to be retained by the double-stranded DNA column are HMG-14 and HMG-17 (Figure 1). All of the HMG proteins were absent in the material retained by the double-stranded DNA column and from the flowthrough of a single-stranded DNA column (not shown).

We have previously reported the apparent absence of HMG-14 and HMG-17 in the single-stranded DNA binding fraction (6), but we now know that our detection of those two proteins was at best sporadic in the one-dimensional polyacrylamide/SDS gel electrophoresis system used in the previous analyses. The current analysis by two-dimensional gel electrophoresis strongly suggests that, like the high molecular weight HMG proteins, HMG-14 and HMG-17 possess a marked preferential affinity for single-stranded DNA in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5).

We have also examined protein fractions from sequential DNA chromatography by acid/urea gel electrophoresis and obtained results (19) that are entirely consistent with those based on the two-dimensional electrophoretic analyses described above. Analysis of material eluted from the single-stranded DNA column by 0.3, 0.4, 0.5, and 2 M NaCl (all in 1 mM Tris-Cl, pH 7.5), showed that HMG-14 and HMG-17 were retained by the column during the wash with 0.2 M NaCl and after application of 0.3 M NaCl, and they were eluted by application of 0.4 M NaCl. Before treatment of the double-stranded DNA column with S1 nuclease, all of the HMG proteins were retained by that column in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5) (reference 19). We have previously reported that for the high molecular weight HMG proteins (6).

DNA cellulose chromatography of purified HMG-14b and HMG-17. It is not inconceivable that the behavior of any individual protein could be influenced during sequential DNA cellulose chromatography by the presence of other proteins in the extract. Hence, a more convincing demonstration of preferential affinity for single-stranded DNA is obtained by chromatography of purified proteins, such as we have recently reported for the high molecular weight HMG proteins (20). We have therefore chromatographed purified HMG-14 and HMG-17 on double-stranded and single-stranded DNA cellulose columns. We have used HMG-14b in these studies since it is more readily obtained than HMG-14a (reference 14).

As demonstrated in the elution profiles shown in Figure 2, HMG-14b was not retained by a double-stranded DNA cellulose column at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5) but was retained by a single-stranded DNA cellulose column under the same conditions. When HMG-14b was applied to the same double-stranded DNA cellulose column before it had been treated with S1 nuclease, all of the

Figure 2. Chromatography of HMG-14b on columns containing immobilized double-stranded DNA (Panel A) and single-stranded DNA (Panel B). The protein samples were applied to the columns in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). Arrows indicate points of application of higher NaCl concentrations (all in 1 mM Tris-Cl, pH 7.5). Fraction volumes, 6.7 ml in Panel A and 6.0 ml in Panel B. Gel electrophoresis of material in fractions 40-43 in Panel A failed to reveal any protein.

protein was retained by that column in 0.2 M NaCl (Figure 4a). Comparable results were obtained by chromatography of purified HMG-17 (Figures 3 and 4b). HMG-14 and HMG-17 were not measurably retarded by the S1 nuclease-treated double-stranded DNA cellulose column; in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5) they eluted from that column in the same volume of solvent as did a sample of NaCl which was chromatographed separately to identify the elution volume of

Figure 3. Chromatography of HMG-17 on columns containing immobilized double-stranded DNA (Panel A) and single-stranded DNA (Panel B). The protein samples were applied to the columns in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). Arrows indicate points of application of higher NaCl concentrations (all in 1 mM Tris-Cl, pH 7.5). Fraction volume, 6.7 ml.

a substance that did not interact with the column. Acid/urea gel electrophoresis of the proteins eluted from the DNA cellulose columns (Figure 5) demonstrated that HMG-14 and HMG-17 were recovered intact from the experiments just described. This is important to verify, since many breakdown products of HMG proteins have very different DNA binding properties than the parent molecules (Isackson and Reeck, manuscript in preparation).

DISCUSSION

The results of DNA cellulose chromatography presented in this paper indicate that HMG-14 and HMG-17 possess a marked preferential affinity for single-stranded DNA at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). Particularly compelling evidence for that comes from chromatography of the purified proteins on double-stranded DNA cellulose columns before and after treatment of the columns with

Figure 4. Chromatography of HMG-14b (Panel A) and HMG-17 (Panel B) on columns that contained double-stranded DNA and that had not been treated with S1 nuclease. Samples were applied in 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). Flow-through material would have appeared from each column at approximately fraction number 18. Fraction volumes, 6.0 ml in Panel A and 7.5 ml in Panel B.

S1 nuclease. Under the conditions in which we circulate S1 nuclease through columns, the enzyme preparation displays a strict preference for single-stranded DNA (K. Shastri and G. R. Reeck, unpublished observations). Treatments with S1 nuclease eliminate the ability of double-stranded DNA columns to retain HMG proteins at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). That is clearly not due to the enzyme's simply removing all of the DNA from the columns, since S1 nuclease-treated columns bind other chromatin proteins at 0.2 M NaCl and the HMG proteins themselves at lower ionic strengths. Further, the treated columns slowly regain their ability to bind HMG proteins at 0.2 M NaCl. Since that ability can once again be eliminated by treatment of the columns with S1

Figure 5. Acid/urea gel electrophoresis of proteins recovered from DNA cellulose chromatography experiments. Gel samples were: Track 1, HMG-14b from fractions 19-24 of Figure 2A; Track 2, HMG-14b from fractions 44-47 of Figure 2B; Track 3, HMG-14b from fractions 42-45 of Figure 4A; Track 4, HMG-17 from fractions 20-25 of Figure 3A; Track 5, HMG-17 from fractions 45-48 of Figure 3B; Track 6, HMG-17 from fractions 37-40, Figure 4B.

nuclease, we assume it is conferred on a column by the action on the DNA in the columns by low levels of contaminating nucleases.

From this and previously published work from this laboratory (6,7,20) it can be concluded that all of the well-defined HMG proteins possess preferential affinity for single-stranded DNA at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5). Since the low molecular weight HMG proteins [HMG-14, HMG-17 and their homologs, such as trout testis H6 (reference 1)] and the high molecular weight HMG proteins (HMG-1, HMG-2, and HMG-E) appear unrelated in amino acid sequence (21-23), protein conformation (24-27), and mode of interaction with nucleosomes (1-5,28) it seems that the two groups of proteins are unrelated structurally or functionally. That the two subsets of HMG proteins both display preferential affinity for single-stranded DNA would therefore appear to be simply fortuitous.

The implications of the HMG proteins' preferential affinities for single-

stranded DNA for understanding the proteins' functions are unclear. HMG-1 and/or HMG-2 appear to be enriched in proliferating cells (7,29). It has therefore been suggested that they might function in DNA replication as helix-destabilizing proteins (7), but there is as yet no direct evidence for that. The preferential association of HMG-14 and HMG-17 with nucleosomes containing transcribed DNA strongly suggests a role of those proteins in transcription, but such a role is again unproven. We would nonetheless like to offer two speculations on the possible roles of the proteins, based on our results.

First, the DNA binding properties of HMG-14 and HMG-17 might be important in the abilities of the proteins to specifically recognize nucleosomes that contain transcribed DNA sequences. That is, the same aspects of the structures of HMG-14 and HMG-17 that result in preferential affinity of the proteins for naked single-stranded DNA over naked double-stranded DNA might also result in preferential association of the proteins with DNA within active nucleosomes over DNA within bulk nucleosomes. Available data appear consistent with the following ranking of four types of DNA according to the strengths with which HMG-14 and HMG-17 bind to them: naked double-stranded DNA < DNA in inactive nucleosomes < DNA in active nucleosomes $\leq$ naked single-stranded DNA. Sandeen et al. (4) have demonstrated directly that HMG-14 and HMG-17 bind more strongly to monomeric nucleosomes than to naked double-stranded DNA. Data of the same authors and of Weisbrod and Weintraub (5) demonstrate that HMG-14 and HMG-17 bind more strongly to active nucleosomes than to inactive nucleosomes. We are left, then, with positioning naked single-stranded DNA in the hierarchy. This requires comparison of results from several laboratories, and we do so with the realization that rather different techniques were involved in the experiments. At NaCl concentrations above 0.1 M, Sandeen et al. (4) observed no interaction of HMG-14 and HMG-17 with naked double-stranded DNA (which is consistent with our results) or with bulk nucleosomes. Since we have found HMG-14 and HMG-17 to require 0.4 M NaCl to elute from a column containing immobilized single-stranded DNA, it would appear that the proteins bind more strongly to naked single-stranded DNA than to DNA in bulk nucleosomes. Gazit et al. (30) reported that the selective digestion of DNA in active nucleosomes by DNase I is largely abolished above 0.3 M NaCl; and Weisbrod and Weintraub (5) found that active nucleosomes were eluted at 0.4 M NaCl from columns containing immobilized HMG-14 and HMG-17. Thus, it would appear that NaCl concentrations in the range of 0.3 - 0.4 M are required to largely or totally disrupt the interactions of HMG-14 and HMG-17 with DNA in

active nucleosomes. (The NaCl concentration required will almost certainly depend upon the concentrations of HMG proteins and nucleosomes or of chromatin used in any particular experiment, since dilution itself will promote dissociation). Since we found HMG-14 and HMG-17 to elute at 0.4 M NaCl from a column containing immobilized single-stranded DNA, it seems that HMG-14 and HMG-17 may bind to naked single-stranded DNA with an affinity equal to or perhaps greater than the affinity with which they bind to DNA in active nucleosomes.

The DNA in nucleosomes is negatively supercoiled and therefore less tightly wound than naked double-stranded DNA (31). If the DNA in actively transcribed nucleosomes were somewhat more negatively supercoiled than DNA in inactive nucleosomes, the ranking given above might simply reflect the extent to which the four types of DNA are unwound. Even if the position we assigned naked single-stranded DNA turns out to be wrong, the marked preferential affinity of the proteins for single-stranded DNA over double-stranded DNA at 0.2 M NaCl indicates that differences in DNA conformation may be sufficient to explain the differences in affinities of HMG-14 and HMG-17 for naked double-stranded DNA, bulk nucleosomes, and active nucleosomes.

Our second speculation is that the preferential affinity of HMG-14 and HMG-17 for single-stranded DNA might allow the proteins to assist in unfolding nucleosomes during transcription. Weisbrod and Weintraub (3) have suggested that HMG-14 and HMG-17 might "open up the nucleosome" or "melt out some of the DNA." Our finding that the proteins bind strongly to single-stranded DNA at roughly physiological ionic strength may be relevant to those suggestions. Sandeen et al. (4) reported that the proteins do not lower the melting temperatures of naked DNA or of DNA in bulk nucleosomes. Presumably there exist kinetic blocks that disallow the proteins from exerting the thermodynamically inevitable consequence of their preferential affinity for single-stranded DNA. [Such a block has been postulated as the basis of the inability of the gene 32 protein to lower the melting temperature of naturally occurring DNA's (30).] Available data do not, however, exclude the possibility that HMG-14 and HMG-17 lower the melting temperature of DNA in active nucleosomes or the possibility that the proteins might facilitate the separation of DNA strands by acting in concert with other proteins, perhaps RNA polymerase.

Finally, we note that the two-dimensional electrophoretogram of Figure 1 suggests that there are other proteins in the 0.35 M NaCl extract of chicken erythrocyte chromatin that possess preferential affinity for single-stranded DNA. Verifying that will require purification of such proteins and study of

their interactions with DNA as individual proteins.

ACKNOWLEDGMENTS

We thank William Debold for excellent technical assistance. This work was supported by the Kansas Agricultural Experiment Station and grants from the National Institutes of Health (R01-CA-17782 and K04-CA-00425). This is contribution number 81-429-j, Kansas Agricultural Experiment Station.

FOOTNOTE

¹Abbreviation used: HMG, High Mobility Group. By the term "low molecular weight HMG proteins" we mean HMG-14, HMG-17 and sequence homologs of those proteins (for example, H6 from trout testis).

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