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DNA intercalators induce specific release of HMG 14, HMG 17 and other DNA-binding proteins from chicken erythrocyte chromatin

Hennrik Schröter, Gernot Maier[1], Herwig Ponstingl[1] and Alfred Nordheim

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, and [1]Institut für Zell- und Tumorbiologie, Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG

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Chicken erythrocyte nuclei were incubated with DNA intercalating agents in order to isolate from chromatin specific DNA-binding proteins whose binding specificity may be determined by DNA secondary and/or tertiary structure. The intercalating agents ethidium bromide (EtBr) and propidium iodide induce the specific release of high mobility group proteins HMG 14 and HMG 17 under low ionic strength conditions. Chloroquine (CQ) intercalation also results in the selective liberation of HMG 14 and HMG 17, but, in addition, selectively releases other nuclear proteins (including histone H1A) in a pH- and ionic strength-dependent fashion. The use of this new 'elutive intercalation' technique for the isolation and purification of 'sequence-specific' and 'helix-specific' DNA-binding proteins is suggested.

Key words: high mobility group proteins/DNA conformation/protein sequencing/nonhistone chromosomal proteins

Introduction

The DNA-protein complexes building up eukaryotic chromatin involve proteins that participate in the structural organization as well as the regulated expression of the genetic material (Igo-Kemenes *et al.*, 1982; Weisbrod, 1982). The most abundant structural proteins are histones, which give rise to nucleosome core formation (involving histones H2A, H2B, H3 and H4) and higher order chromatin structure (involving additional histones such as H1, H5). The class of nonhistone chromosomal proteins contains highly heterogeneous members, of which the most abundant ones are the high mobility group (HMG) proteins (for review, see Johns, 1982).

The DNA component of chromatin represents a structurally flexible macromolecule, as evidenced by crystallographic studies of the nucleosome core particle (Richmond *et al.*, 1984) or a procaryotic DNA-protein complex (Frederick *et al.*, 1984). In addition, helix unwinding, strand unpairing, DNA bending, formation of cruciform structures and generation of left handed Z-DNA are changes within the DNA secondary structure that have been biochemically characterized *in vitro* (Zimmermann, 1982; Rich *et al.*, 1984). The double helical DNA conformation is especially sensitive to the presence of topological stress within underwound, negatively supercoiled DNA domains (Wang *et al.*, 1983). At present it is unknown to what extent such states of DNA secondary and tertiary structure participate in establishing chromatin structure *in vivo*. The degree to which polymorphism in DNA structure is directly involved in the generation of specific DNA-protein interactions within chromatin is also difficult to assess. A search for DNA-binding proteins which selectively recognize DNA conformational states would address this issue.

The structures of DNA, both secondary and tertiary, are strongly affected by DNA intercalating agents. The most striking examples are the unwinding of B-DNA and the destabilization of Z-DNA (Pohl *et al.*, 1972). Unwinding of B-DNA within a topologically constrained domain results in the gradual reduction of negative superhelical stress followed by the introduction of positive superhelical turns.

To probe for the effects that intercalators exert on chromatin, we incubated nuclei of chicken erythrocytes with DNA intercalating agents (ethidium bromide, propidium iodide, chloroquine) and monitored the release of chromosomal proteins by gel electrophoresis. We found that the high mobility group proteins HMG 14 and HMG 17 are selectively liberated from chromatin upon addition of DNA intercalating agents. The degree of specificity in the release of HMG proteins 14 and 17 could be modulated by choosing different ionic strength conditions. The type and concentration of the intercalator used also affect the profile of eluted proteins. The protein extracts obtained by this technique of 'elutive intercalation' were functionally characterized with respect to DNA-binding affinity, nuclease contamination and content of HMG protein.

'Elutive intercalation' provides a new technique for (i) rapidly purifying HMG 14 and HMG 17 proteins, (ii) potentially isolating DNA-binding proteins that exhibit binding specificity for DNA conformation and, more generally, (iii) for probing specific DNA-protein interactions in eukaryotic nuclear chromatin.

Results

Nuclear protein elution caused by the intercalator ethidium bromide

Incubation of nuclei from adult chicken erythrocytes with the DNA intercalating agent ethidium bromide (EtBr) induces the selective release of chromosomal proteins. These proteins can be recovered by pelleting the nuclei followed by 20% trichloroacetic acid (TCA) precipitation of the resulting supernatants. SDS-polyacrylamide gel electrophoretic (PAGE) analysis (Laemmli, 1970) of such TCA precipitates (Figure 1, lanes 1–3), reveals the presence of two major protein bands. Increasing amounts of these two proteins are recovered when increasing concentrations of intercalator are applied in the range of 5–10 mM. These two major proteins co-migrate electrophoretically with the high mobility group proteins HMG 14 and HMG 17 as revealed by simultaneous electrophoresis of total histone preparations for chicken erythrocyte nuclei (Figure 1, lane H) and total HMG preparations (Figure 1, lane HMG) (see below). Lanes 1–3 of Figure 1 also reveal the presence of minor components in the protein extracts, including some intercalation-unrelated protein bands (see below). The eluted proteins are virtually free of core histones.

When EtBr concentrations >10 mM are used to incubate nuclei, the eluted protein bands co-migrating with HMG 14/17

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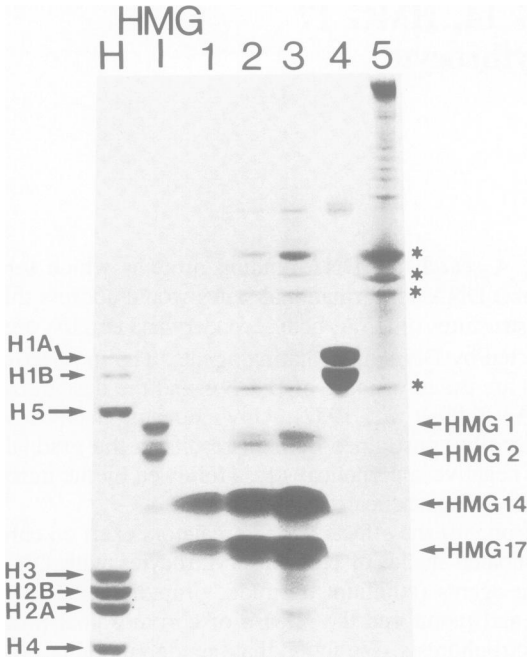

Fig. 1. Release of HMG 14, HMG 17 and histones H1A, H1B upon intercalation of chromatin with EtBr. 3.6×10^8 nuclei of adult chicken erythrocytes were incubated with increasing amounts of EtBr (**lanes 1–4**), the nuclei were then pelleted and the protein contents of the supernatants were concentrated by 20% TCA precipitation and separated by SDS-PAGE. EtBr concentrations used in **lanes 1–4** were 5.0, 7.5, 10 and 20 mM, respectively. Incubation was performed under low salt buffer conditions (buffer X2). **Lane 5** shows the control experiment in which 1.8×10^9 nuclei were incubated in the absence of intercalators and under the less stringent buffer E conditions (see text). Asterisks emphasize four of the intercalation-unrelated protein bands which are released under these conditions. Lanes labelled H and HMG show banding positions of total histone and total HMG proteins, respectively, as prepared from adult chicken erythrocyte nuclei. Identifications of histone and HMG proteins are given on the outer margins. The gels were stained with Coomassie blue to visualize protein material.

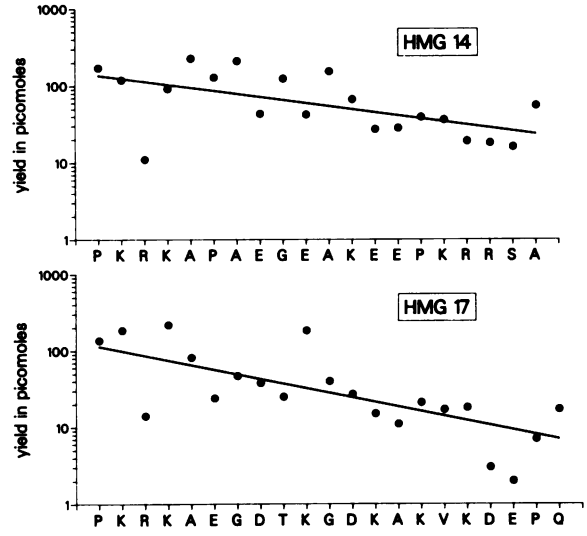

Fig. 2. Identification of intercalator released HMG 14 and HMG 17 proteins by microsequencing analysis. Yield (in pmol) of phenylthiohydantoin derivatives is plotted over the obtained amino acid sequence.

are gradually reduced in intensity, while at the same time two new bands appear which co-migrate with histones H1A and H1B (data not shown). The latter two proteins are the most prominent species released by 20 mM EtBr (Figure 1, lane 4). It is unclear whether the diminished release of HMG 14/17 reflects a specific response to intercalator-induced rearrangement of chromatin.

The observed enrichment of HMG 14/17 proteins in the supernatant fractions of lanes 1–3, Figure 1, is not due to selective precipitation during sample preparation, since lyophilization not involving TCA precipitation yields identical protein recovery (not shown). The specificity of the release of HMG 14, HMG 17 and the putative H1A/H1B histones is further supported by the control experiment shown in Figure 1, lane 5. A five times higher number of nuclei was incubated in the absence of intercalating agent and the released proteins were processed in an identical manner to the preparations in Figure 1, lanes 1–4. Under these conditions a number of proteins appear to leak out of the nuclei. The appearance of these unspecific proteins is unrelated to addition of DNA intercalators and these proteins, of which four prominent ones are marked in lane 5 with asterisks, do not co-migrate with any of the specific intercalator-released proteins. The control in lane 5 also employed a buffer of slightly higher ionic strength (buffer E), generating conditions of lower stringency with respect to intercalator-induced protein release. Incubation of nuclei in buffer E containing 10 mM EtBr releases some histone H1A in addition to HMG 14 and HMG 17; see lane 1, Figure 4A. The intercalation-unrelated protein bands could not be identified unambiguously.

The high degree of selectivity, as exhibited by EtBr-induced release of HMG 14 and HMG 17, is especially apparent if one takes into account the relatively lower abundance in the nucleus of these two proteins compared with HMG 1 and HMG 2 (see Figure 1, lane HMG) or total histone proteins.

Microsequencing of HMG 14 and HMG 17 proteins

To identify unambiguously the nature of the HMG 14 and 17 proteins released by the intercalator, microsequencing analysis of gel electrophoretically purified material was performed. The sequence of the amino-terminal region was determined for both proteins (Figure 2) and was found to be identical to the published amino acid sequences of chicken HMG 14 and 17 proteins (Walker *et al.*, 1980; Walker and Johns, 1980).

The DNA intercalators chloroquine and propidium iodide also induce release of chromatin proteins

To investigate further the mechanism of the EtBr-induced protein release, we analyzed the effects which another DNA intercalator, chloroquine (CQ), exerts on the stability of DNA-protein complexes within chromatin. Figure 3A, lanes 1–3, shows the gel electrophoretic analysis of proteins obtained upon incubation of nuclei in low salt buffer X2 in the presence of 7.5, 10 and 20 mM CQ, respectively. As with EtBr, CQ also induces selective release of chromatin proteins. It is apparent, however, that, under these conditions, a broader spectrum of proteins is released. Proteins liberated by CQ include species co-migrating with HMG 14, HMG 17, H1A, HMG 2 and a range of proteins of lower electrophoretic mobility. The absence of the abundant nuclear proteins H1B, H5, HMG 1 and core histones again reveals a considerable degree of selectivity in the protein release. For a direct comparison between EtBr and CQ, the protein elution profile obtained with EtBr under identical conditions is shown in lane 4 of Figure 3 (this sample is identical to lane 4, Figure 1). Although the degree of specificity in protein release differs

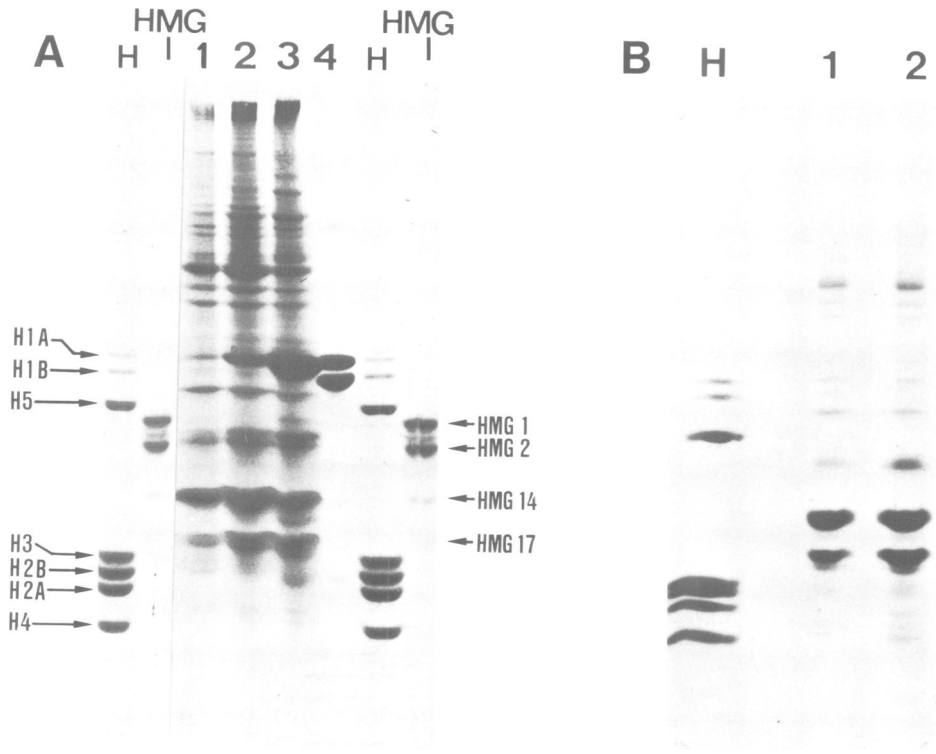

Fig. 3. Release of nuclear proteins upon intercalation of chromatin with chloroquine or propidium iodide. (**A**) CQ (pH 7) at concentrations of 7.5, 10 and 20 mM was applied to chicken erythrocyte nuclei (as described in Figure 1) and the proteins found in the resulting supernatants were electrophoresed in **lanes 1, 2** and **3**, respectively. **Lane 4** contains the material from Figure 1, lane 4, for comparison of protein release profiles of the two intercalators CQ and EtBr. H and HMG denote lanes of total histones and total HMG proteins, respectively. (**B**) Nuclear extracts obtained upon incubation with propidium iodide at 2.5 mM (**lane 1**) or 5 mM (**lane 2**) final concentration. Lane H contains the preparation of total histones.

between EtBr and CQ extraction, the relatedness of the elution profiles nevertheless suggests that DNA intercalation is instrumental in the liberation of the selected subset of chromatin-bound proteins.

We also incubated chicken erythrocyte nuclei with a third intercalator, propidium iodide. As shown in Figure 3B, lanes 1 and 2, propidium iodide at concentrations of 2.5 and 5 mM, respectively, also causes the nuclear release of two major protein bands. Again these two bands comigrate with HMG proteins 14 and 17, generating a profile of eluted proteins that is very similar to the profile generated by EtBr in the range between 5 and 10 mM.

It is possible that binding of DNA intercalating drugs to proteins accompanied by changes in protein conformation could potentially have caused the observed specific protein elution release of HMG proteins 14 and 17. To test for this possibility, nuclei were incubated with radioactive CQ ([^{3}H]CQ, New England Nuclear) at a total CQ concentration of 10 mM. The extract obtained was chromatographed on a Sephadex G25 sizing column to separate proteins from free CQ. No CQ was found associated with the extracted proteins after this chromatography step (data not shown). We, therefore, could not observe a high binding affinity for CQ to the extracted proteins. Although this finding does not completely rule out the possibility of intercalator binding to chromatin-associated proteins inside the nuclei it nevertheless suggests that interactions between proteins and intercalators are not likely to be the primary cause for the observed release of HMG proteins 14 and 17 from chromatin.

The effect of pH and associated changes in ionic strength on CQ-induced protein release

Chloroquine diphosphate, when present in the 5 – 20 mM concentration range, lowers the pH of Tris-buffered solutions. Therefore, in the experiments described in Figure 3A, care was taken to adjust the pH to neutrality before incubation of nuclei with CQ solutions. If, however, nuclei are incubated in buffer E, having a pH of 5.6 upon addition of 10 mM CQ, the resulting protein elution profile contains only a restricted range of protein species, mainly consisting of HMG 14 and HMG 17 (Figure 4A, lane 3). This profile must be contrasted to the protein profile obtained with 10 mM CQ in buffer E at pH 7.4 (Figure 4A, lane 2). Figure 4A, lane 1, shows, for comparison, the proteins released by 10 mM EtBr (buffer E, pH 7.4). (Note that 10 mM EtBr induces the release of histone H1A in buffer E, but not in buffer X 2; Figure 1, lane 3, and Figure 4A, lane 1.) It appears that at low pH CQ displays an enhanced degree of selectivity with regard to the release of HMG 14 and HMG 17 proteins.

To further investigate this pH effect, nuclei were incubated with 10 mM CQ at an increasing range of pH values. Figure 4B, lanes 1 – 6, shows the protein components released at pH values of 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, respectively. With increasing pH, increasing amounts of HMG 14 and 17 proteins are liberated while concomitantly the overall spectrum of eluted proteins becomes more complex. The increase in histone H1A elution towards neutral pH conditions is especially apparent. It has to be pointed out that adjustment of pH values is associated with subtle changes in ionic strength which are expected to contribute to the observed protein release profiles.

Additive destabilization of chromatin DNA-protein complexes by EtBr and NaCl

To obtain information about the influence of ionic strength conditions on the intercalator-induced protein release, nuclei were incubated with 7.5 mM EtBr (in buffer X2) in the presence of

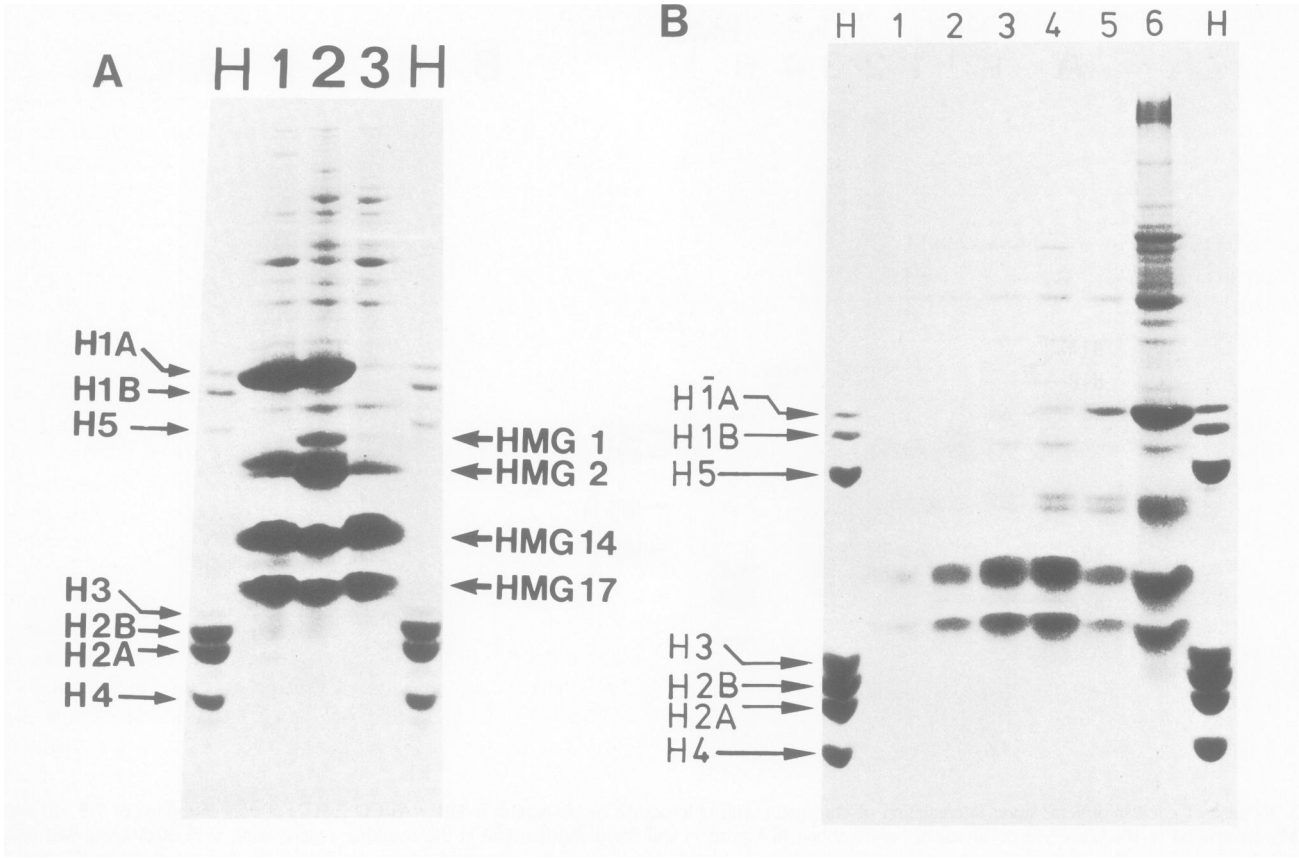

Fig. 4. Effect of pH adjustment on CQ-induced protein release profiles. (**A**) EtBr (10 mM/pH = 7.4, **lane 1**) and CQ (10 mM/pH = 7.4, **lane 2**; 10 mM/pH = 5.6, **lane 3**) were used to incubate nuclei. The reduced complexity of the protein elution profile, as obtained by low-pH CQ intercalation, becomes apparent. (**B**) **Lanes 1–6** exhibit the protein species released after intercalation of nuclei with CQ at the increasing pH values of 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, respectively. To achieve stable pH values the following buffers (1 mM) were used: Na-citrate (pH = 5.0), MES (pH = 5.5, 6.0, 6.5) and Hepes (pH = 7.0, 7.5). Lanes H show total histone separation profiles.

increasing NaCl. Figure 5 displays the protein elution profiles obtained when 0, 5, 10, 25, 50, 100 and 150 mM NaCl (lanes 1–7, respectively) were added to the buffered intercalator solution during incubation of the nuclei. Whereas no appreciable amounts of additional HMG 14 and 17 proteins are released at higher ionic strengths, other proteins are clearly affected. These include two protein bands that co-migrate with the two forms of HMG 2, histone H1A, as well as with some high mol. wt. protein material (the protein bands marked with asterisks; the appearance of which is unrelated to addition of intercalators, also increase in intensity). Extending this NaCl titration to 350 mM NaCl, the intercalated chromatin appears to collapse, since under these conditions core histones are also released (data not shown). The lack of additional release of HMG 14 and HMG 17 upon addition of NaCl suggests that at 7.5 mM EtBr these HMG proteins are released in near quantitative amounts. A comparable experiment using 10 mM CQ with increasing amounts of NaCl exhibits some additional release of HMG 14 and HMG 17 compared with the NaCl-free conditions (data not shown). This finding coincides with our experience that, by comparison, CQ is a somewhat less effective intercalator than EtBr with respect to the liberation of HMG 14/17 proteins.

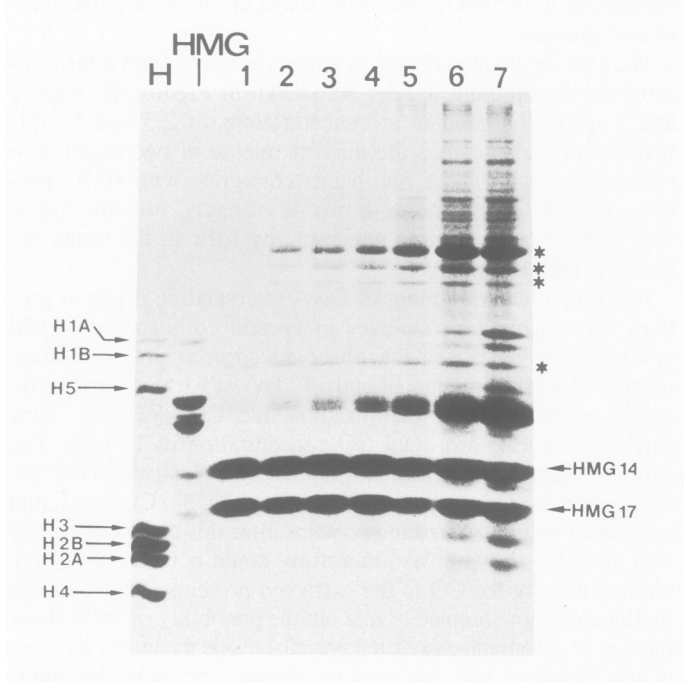

Fig. 5. Quantitative release of HMG 14 and HMG 17 by 7.5 mM EtBr. Nuclei were incubated with 7.5 mM EtBr either in the absence (**lane 1**) or in the presence (**lanes 2–7**) of increasing concentrations of NaCl. NaCl concentrations (mM) in **lanes 2–7** were 5, 10, 25, 50, 100 and 150, respectively. H and HMG are total histone and total HMG control lanes, respectively.

Discussion

Specific DNA-protein interactions are determined to a significant degree by the geometry and structure of the double-helical DNA molecule (Pabo and Sauer, 1984). The structure of DNA

itself can be affected drastically upon binding of DNA-intercalating agents (see, for example, Pohl *et al.*, 1972). Consequently, DNA intercalators could be shown to have a strong effect on the stability of DNA-protein complexes (see, for example, Strätling and Seidel, 1976).

In the present work we have tested the possibility of selective protein release from eucaryotic chromatin upon incubation of nuclei with DNA intercalating agents. Specific protein release could be achieved with the three different DNA intercalators, EtBr, propidium iodide and CQ. It is therefore likely that DNA intercalation itself causes the observed liberation of DNA-binding proteins. We would like to refer to the described new approach of generating nuclear extracts using DNA intercalating agents as 'elutive intercalation'.

As judged by visual inspection of SDS-acrylamide gels stained with Coomassie blue, protein release was achieved most efficiently with the high mobility group proteins HMG 14 and HMG 17, as well as with histone H1A at higher concentrations of intercalator. Under these conditions no release of core histones, histone H5 or high mobility group protein HMG 1 was observed. This high degree of specificity in the intercalation-induced protein elution profile is especially significant if one considers the lower intranuclear mass of HMGs 14 and 17 compared with HMGs 1 and 2 or histone proteins. The classical way of releasing HMG proteins involves salt extraction, whereby, upon gradual increase in salt concentration up to 0.35 M, HMG 1 and HMG 2 are released before HMG 14 and HMG 17 (Schröter, 1982). In contrast, as shown here, DNA intercalating agents release HMG 14 and 17 almost quantitatively while the nuclear content of HMG proteins 1 and 2 is released only marginally. The extracts obtained by 'elutive intercalation' exhibit distinct DNA-binding activity which was strongly ionic strength-dependent; the presence of increasing NaCl in the range of 120 – 160 mM abolished DNA-binding, as assayed by nitrocellulose filter binding assays (H.Schröter and A.Nordheim, unpublished). An EDTA-sensitive endonucleolytic DNA cleavage activity was also found in the extracts (not shown).

Although both EtBr and CQ caused release of HMG 14 and HMG 17, the two intercalators nevertheless exhibited somewhat different characteristics. EtBr was invariably more efficient than CQ with respect to protein release at a fixed concentration of intercalator. CQ, on the other hand, gave rise to a broader spectrum of released proteins when used at neutral pH. The protein elution profile of both intercalators can be modulated greatly by the ionic conditions applied during intercalation (see Figures 4 and 5).

It should be pointed out that the concentrations of intercalator required for generating the effects described ranged from 1 to 20 mM. This appears to be a disconcertingly high value. However, for the time being we do not have good estimates of the actual extent of DNA intercalation achieved by incubation of whole nuclei.

The most obvious effect produced by 'elutive intercalation' is release of HMG 14 and HMG17 (Figures 1, 4 and 5). HMGs represent a class of abundant nonhistone chromosomal proteins which are present in animals, plants and fungi (Spiker *et al.*, 1978). HMGs are characterized by their extractability from chromatin by 0.35 M NaCl, their solubility in 2% TCA, their high mobility in polyacrylamide gel electrophoresis and their amino acid composition being high in both basic and acidic amino acids. It has been suggested that these are localized in the internucleosomal linker region of chromatin (HMGs 1 and 2) and in the core particle DNA exit regions (HMGs 14 and 17) (Levy-Wilson *et al.*, 1977; Sandeen *et al.*, 1980; Schröter and Bode, 1982). Post-synthetic modifications of HMG proteins include methylation, acetylation, phosphorylation, poly(ADP)-ribosylation and glycosylation (Allfrey, 1982). Preferential release of the H6 HMG protein was achieved by limited nuclease digestion (DNase I) of actively transcribed chromatin segments from trout testis nuclei (Levy-Wilson *et al.*, 1980). Functionally, HMG 14 and HMG 17 have been implicated in structural stabilization of nucleosome core particles (Sandeen *et al.*, 1980; Paton *et al.*, 1983) as well as in conferment of DNase I sensitivity to potentially active chromatin segments (Weisbrod and Weintraub, 1979). The actual *in vivo* functions of HMG proteins, however, are still not understood.

The reasons for specific release of HMG 14, 17 by 'elutive intercalation' are not clear. The interactions of HMG proteins with chromatin are thought to be of ionic nature (Watson *et al.*, 1979). Since we achieve specific release of HMG 14 and 17 using intercalators in low ionic strength buffers (X2, TEA) reasons other than weakening of ionic interactions must contribute to generating the observed effect. Structural changes of chromatin as consequences of DNA intercalation are postulated to be involved. Contributing factors may include preferential intercalation at nucleosomal HMG 14 and HMG 17 binding sites, intercalator-induced alterations of protein-protein interactions between core histones and HMG proteins 14 and 17 or even preferred HMG binding to DNA double-helical conformations that are sensitive to DNA intercalation. The highly speculative possibility that there is preferential DNA intercalation into chromosomal segments whose chromatin structure is altered by binding of HMG 14 and HMG 17, cannot be ruled out completely. However, the almost quantitative removal of HMGs 14 and 17 obtained in conditions in Figure 5 argues strongly against this speculation.

'Elutive intercalation' may prove a useful technique to investigate functional aspects of HMG proteins. Providing a convenient method for preparation of HMG proteins 14 and 17, this method can additionally be used in screening species- and cell type-specific distribution of HMG proteins. The distribution and chromatin-binding characteristics of different post-translationally modified HMG derivatives can also be studied with this technique. Furthermore, a better understanding of DNA intercalation within chromatin may help elucidate the exact location of HMGs 14 and 17 with respect to transcriptionally active and inactive chromatin.

Finally, DNA intercalators may be useful in searching for naturally occurring forms of altered DNA secondary and tertiary structure. Since DNA intercalators change the geometry of right-handed B-DNA, revert left-handed double helices and also relieve negative torsional stress, associated effects on the stability of specific DNA-protein interactions may be revealed by 'elutive intercalation'. Consequently, this technique may facilitate the search for both sequence-specific' and 'helix-specific' eucaryotic DNA-binding proteins. We are currently investigating the usefulness of this technique for purification of regulatory DNA-binding proteins. The nuclear extracts generated by 'elutive intercalation' are also being used in our search for Z-DNA-binding proteins (Nordheim *et al.*, 1982).

Materials and methods

Buffers

Buffer X2 (1 mM Tris-HCl, pH = 7.0, 0.2 mM EDTA). Buffer E (10 mM Tris-HCl, pH 7.0, 5 mM MgCl, 2 mM CaCl, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM β-mercaptoethanol) (for preparation and storage of nuclei this

buffer was adjusted to pH = 7.4). Buffer TEA (1 mM triethanolamine, pH = 7.5, 0.2 mM EDTA).

Preparation of nuclei from chicken erythrocytes

Four litres of chicken blood from a local slaughter house were collected in 1/5 volume of 75 mM NaCl, 25 mM EDTA, pH 7.5. The cells were washed and nuclei were released by incubation with 5% Triton in buffer E, washed extensively in this buffer containing 0.35 M sucrose, and quick frozen in liquid nitrogen after addition of dimethylsulfoxide (DMSO) to 7.5%. Nuclei were stored at −70°C.

'Elutive intercalation' extracts

For analytical protein extraction by elutive intercalation, nuclei were first washed in buffer E and then in the buffer of choice. In the case of low ionic strength buffers (TEA or X2), nuclei were adjusted by dropwise addition of 9 volumes of low ionic strength buffer, sedimented at 700 *g* for 20 min and resuspended in low ionic strength buffer. Intercalator was added to nuclei (1.8×10^8/ml) to the indicated concentrations (stock solutions: 0.5 M chloroquine diphosphate, 25 mM EtBr, 20 mM propidium iodide) and incubation at 0°C was allowed for 30 min with or without shaking in the case of buffer E or low salt buffer, respectively. All intercalators were obtained from Sigma Chemie GmbH (Munich).

Nuclei were sedimented at 700−2000 *g* for 20 min. The collected supernatant was clarified by centrifugation (4000 *g*, 10 min). For analytical studies the extract was adjusted to 20% TCA and after overnight incubation at 0°C precipitated proteins were collected by centrifugation (5000 *g*, 20 min). The protein pellet was washed three times with acetone/0.1 N HCl (7:1) and once with acetone before adding SDS-loading buffer for electrophoresis (Laemmli, 1970). Proteins eluted from 3.6×10^8 nuclei were analyzed per lane on a 13% acrylamide gel (acrylamide:bisacrylamide, 30:0.8), stained with Coomassie brilliant blue G 250 in 10% TCA and destained in 7.5% acetic acid. As an alternative procedure, proteins were separated from the intercalator by gel filtration (Sephadex G25, Pharmacia) and the protein solution was dried under vacuum before adding loading buffer for SDS electrophoresis.

For preparative isolation of proteins by elutive intercalation, 9×10^9 nuclei were eluted in 50 ml of 10 mM chloroquine diphosphate in buffer E without readjustment of the pH (final pH 5.6). Concentration of extracted proteins was performed in an Amicon microfiltration system 8 MC with YM2 membrane (Amicon Corp., Danvers) and the final extract was either stored at −70°C or in 50% glycerol at −20°C.

Characterization of extracts

DNA binding activity of the extract was assayed by nitrocellulose filter binding of DNA-protein complexes using tritium-labelled pGF3 plasmid DNA (Nickol and Felsenfeld, 1983). Binding reactions were performed in 5 mM Tris-HCl (pH = 8.0), 37.5 mM NaCl, 50 μg/ml bovine serum albumin and 5 mM EDTA. Retention on filters of complexed [^{3}H]DNA was quantitated by scintillation counting of nitrocellulose filters (Millipore HA filtration plates or Schleicher and Schüll BA 85 membrane filters). To assay for the presence of endonuclease activities, 0.4 μg of pUC18 plasmid DNA (Yannisch-Perron *et al.*, 1985) were incubated (60 min, 37°C) with 1 μg of extracted protein. Incubation buffer was 20 mM Tris-HCl (pH 8.0)/10 mM NaCl. After digestion with proteinase K (50 μg/ml), extraction with phenol and ethanol precipitation, the resuspended DNA samples were analyzed for their topology by electrophoresis in 1% agarose gels.

Identification of HMG proteins 14 and 17 by microsequencing

7×10^9 nuclei were extracted with 40 ml of TEA buffer containing 0.2 mM PMSF and 10 mM β-mercaptoethanol (pH = 7.5). The extract was processed as described above, whereby, after SDS-acrylamide electrophoresis the protein bands were cut out of the gel and electroeluted according to Hunkapiller *et al.* (1983a). Electroeluted samples were dried under vacuum, washed three times with acetone/HCl, 0.1 N (7:1), washed once with acetone, dried and subsequently used for amino acid sequence analysis. Automated Edman degradations were performed with 250 pmol of protein on a gas phase sequencer (Applied Biosystems: Hunkapiller *et al.*, 1983b). Phenylthiohydantoin derivatives of amino acids were identified by h.p.l.c. (LKB, u.v. detector from Kratos), on a Lichrocart superspher RP8 column (Merck, Darmstadt) using an isocratic elution buffer of 68.5% (v/v) 21 mM sodium acetate (pH 4.9), 31.5% acetonitrile plus 0.5% dichloroethane (Lottspeich, 1980).

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