Urea-induced binding of histone 1 to nucleosomes lacking linker DNA

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ABSTRACT

The binding of H1 (and H5) to nucleosome core particles was demonstrated by separating mononucleosomes according to their DNA size on acrylamide gels containing high molarity urea. The presence of urea causes a redistribution of H1 so that it associates with some particles of all linker lengths, including no linker. When the urea is removed the H1 remains associated with particles of all DNA sizes if the different size classes are not mixed with each other. Therefore, urea can effect the transfer of H1 from particles with linker to particles with no linker. When nucleosomes of uniform DNA fragment length, some containing and some lacking H1, are re-electrophoresed under native conditions, they migrate as two widely separated bands. The mobilities of these variants do not depend on linker length and are identical to the mobilities of native H1-containing and H1-lacking particles. When the same collection of particles is electrophoresed in the presence of high molarity urea they migrate with a uniform mobility. These results suggest that H1-containing nucleosomes are conformationally different from H1-lacking particles, but that this difference is eliminated when histone-histone interactions are disrupted by urea.

INTRODUCTION

When chromatin is digested with micrococcal nuclease the "spacer" or linker DNA between the nucleosomes is cleaved and shortened (1-3). In the early stages of digestion oligonucleosomes are released along with a population of mononucleosomes containing DNA of between 140 and approximately 200 base pairs in length. When these mononucleosomes are examined for the presence of histone 1 (H1) it is found that only and all those particles with DNA greater than the fixed length of about 160 base pairs contain this protein (1-3). This phenomenon has given rise to the idea that linker DNA is an important site of H1 binding in chromatin and that a minimum linker length is necessary for the protein-subunit interaction (reviewed in reference (4)).

Recently, however, Gaubatz and Chalkley (5) have demonstrated the binding of H1 to mononucleosomes with an average DNA size of about 150 base pairs (linker length $\sim$ 10 base pairs) and Bonner (6) has presented evidence that H1

can be crosslinked to at least one of the intranucleosomal histones. These data raise the possibility that there is a binding site for H1 on the core particle itself. In this report we show directly that H1 can bind to linkerless core particles. Since the electrophoretic mobility of the linkerless, H1-containing particles is virtually identical to that of H1-containing particles with up to 70 base pairs of spacer, but distinct from that of particles lacking H1, our data imply that H1 binds to the nucleosome similarly in the presence and absence of linker. Finally, the conditions under which transfer of H1 between linker-containing and linkerless nucleosomes occurs suggest that conformational changes in the core particle and/or H1 are involved in their interaction.

MATERIALS AND METHODS

(a) Preparation of Liver Nuclei

Livers were excised from 17 day old chick embryos and washed extensively with Simm's buffered saline solution (7). Five volumes of nuclei preparation buffer (NPB: 0.01 M NaCl, 3 mM CaCl_2 , 0.34 M sucrose, 1 mM phenylmethyl sulfonylfluoride, 0.5% NP-40, 0.01 M tris pH 7.4) were added and the tissue homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was filtered through 20 micron Nytex monofilament nylon mesh, layered over one third volume of 0.1 M NaCl, 3 mM CaCl_2 , 1.37 M sucrose, 1 mM phenylmethyl sulfonylfluoride, 0.01 M tris pH 7.4, and centrifuged at $16,000 \times g$ for 15 minutes. The resulting pellet was resuspended in 7.5 ml of 0.01 M NaCl, 3 mM CaCl_2 , 2.1 M sucrose, 1 mM phenylmethyl sulfonylfluoride, 0.01 M tris pH 7.4, layered over an equal volume of the same solution, and centrifuged for 45 minutes at 24,000 rpm in a Beckman SW27 rotor. The pellet was resuspended in NPB without NP-40 and centrifuged at $500 \times g$ for 10 minutes. The nuclear pellet was resuspended in 5 mM NaCl, 1.5 mM CaCl_2 , 0.17 M sucrose, 1 mM phenylmethyl sulfonylfluoride, 5 mM tris pH 7.4, 50% glycerol and stored at -20°C .

(b) Preparation of Erythrocyte Nuclei

Blood was collected from 17 day old chick embryos, washed several times in Simm's buffered saline solution, and filtered through 20 micron Nytex monofilament nylon mesh. Nuclei were prepared by washing the erythrocytes several times alternately in RSB (0.01 M tris pH 7.4; 0.01 M NaCl; 3 mM MgCl_2) containing 0.5% NP-40 and RSB without detergent (8). Nuclei were stored in RSB and 50% glycerol at -20°C .

(c) Polyacrylamide Gel Electrophoresis of Deoxyribonucleoproteins

Liver and erythrocyte nuclei were digested in 1 mM CaCl_2 , 10 mM Na borate

(pH 7.6); 1 mM CaCl_2 , 10 mM triethanolamine (TEA) pH 7.6; or, 1 mM CaCl_2 , 1 mM TEA (pH 7.6) for 10 minutes at 37°C . Liver nuclei were digested at a concentration of 74 A_{260} units per ml with 800 units per ml micrococcal nuclease (Worthington). Erythrocyte nuclei were digested at a concentration of 148 A_{260} units per ml with 1200 units per ml of the same enzyme. Digestions were stopped by adding an equal volume of 20 mM EDTA, 20% glycerol. A_{260} units for nuclear preparations were determined in 0.1 M NaOH.

Nuclear digests were clarified by centrifugation at $4000 \times g$ for 10 minutes. If digests were pretreated with urea, a solution of 10 M urea (ultra pure grade, Schwarz / Mann), 10 mM EDTA (pH 7.6) was added to give the desired urea concentration and samples were incubated for 15 minutes at 37°C . Samples of 10–20 λ were loaded onto 5% polyacrylamide slab gels with an acrylamide: methylene bis ratio of 30:1 and a buffer system consisting of 2 mM EDTA, 10 mM TEA (pH 7.6) (1). Urea gels were prepared by adding urea to the gel ingredients in the correct molarity. Gels were stained with ethidium bromide (2 $\mu\text{g}/\text{ml}$) for 10 minutes, destained in distilled water, photographed under ultraviolet illumination, and subsequently stained for protein with Coomassie Brilliant Blue. Densitometer tracings were taken on a Photovolt densitometer.

(d) Polyacrylamide Gel Electrophoresis of DNA

DNA from a total nuclear digest was obtained by adding sodium dodecyl sulfate (SDS) to 0.2% to the digestion stopping buffer and digesting for 15 minutes with 2.5 mg/ml proteinase K. The deproteinized digests were loaded onto 5% acrylamide gels as described (1). DNA was isolated from mononucleosome bands by cutting ethidium stained bands into quarters and soaking them in 0.5% SDS, 3 mM EDTA at $\sim 20^\circ\text{C}$ overnight. The extract from the bands was made 1.5 M in NaCl, cooled on ice for 10' and centrifuged at $10,000 \times g$ for 10 minutes (1). After dilution with three volumes of distilled water the supernatant was precipitated with two volumes of ice cold ethanol and centrifuged at $10,000 \times g$ for 20 minutes. The pellet was dissolved in digestion stopping buffer containing 0.2% SDS and loaded onto 5% polyacrylamide gels as described above. Hae III restriction fragments of ϕX DNA were used as molecular weight standards (9).

(e) Polyacrylamide Gel Electrophoresis of Protein

Mononucleosome bands were excised from the gel and incubated in 0.1 M dithiothreitol, 2% SDS, 10% glycerol, 0.12% bromophenyl blue, 0.08 M tris (pH 6.8), for 20 minutes at 50°C . Three identical bands and the buffer in which they had been incubated were loaded onto a 15% SDS polyacrylamide gel. The

gel system used was that of Laemmli (10) with the acrylamide: methylene bis acrylamide ratio modified according to Blattler, et al. (11). Gels were stained according to Laemmli (10).

RESULTS

Isolation of Nucleosome Variants and Determination of their Composition

Digestion of chromatin from several different cell types with micrococcal nuclease, followed by low ionic strength electrophoresis, displays at least two distinct mononucleosome and several oligonucleosome subpopulations (1,12). We have chosen to apply this procedure to nuclei from adult series chicken erythrocytes and chicken liver, with results similar to those reported by Varshavsky, et al. (1). Figure 1 shows the material released into mono- and oligonucleosomes when 2% of the chromatin DNA is rendered acid-soluble by micrococcal nuclease. At this level of digestion subnucleosome nucleoprotein particles (12) have not yet begun to appear. Of the two major mononucleosomes

Figure 1. Polyacrylamide Gel Electrophoresis of Deoxyribonucleoproteins. Total 2% acid soluble nuclease digests of erythrocyte (a,b,e,f) and liver (c, d,g,h) nuclei were electrophoresed on a 5% gel. All samples are on the same gel, which was first stained with ethidium bromide (a-d) and then with Coomassie Blue (e-h). Low mobility and high mobility mononucleosomes are designated by LMM and HMM, respectively.

variants the low mobility species is present in somewhat greater amounts than the high mobility species. Though the slower species often resolved into two bands in our preparations (called MN_2 and MN_3 by Bakayev, et al. (12)) we treated these as a single component in our studies. The material loaded onto the gel was released from the nuclei by treatment with 10 mM EDTA, and comprised 12-25% of the A_{260} present in the undigested nuclei.

By excising the bands containing the two mononucleosome variants from a gel like that pictured in Figure 1, and eluting their material into appropriate buffers, we recovered both the DNA and protein comprising these particles. Figure 2 shows the DNA fragments obtained from each variant, as compared with the "ladder pattern" of DNA fragments obtained by electrophoresing a deproteinized micrococcal nuclease digest of each nuclear preparation. It is clear that the two collections of fragments obtained from each type of nucleus exhibit little overlap in size, and together span the continuum of DNA sizes

Figure 2. DNA Sizes of High and Low Mobility Mononucleosome Populations. Mononucleosome bands were excised from an ethidium bromide stained gel similar to that in Figure 1. Material was deproteinized as described in Materials and Methods. (a) Marker (*Hae* III digested ϕ X DNA); (b) total deproteinized nuclease digest of liver nuclei; (c) DNA from low mobility liver mononucleosomes; (d) DNA from high mobility liver mononucleosomes; (e) DNA from low mobility erythrocyte mononucleosomes; (f) DNA from high mobility erythrocyte mononucleosomes; (g) total deproteinized nuclease digest of erythrocyte nuclei.

characteristic of the mononucleosomes of each cell type.

The proteins comprising the two mononucleosome variants of erythrocyte and liver nuclei were displayed by electrophoresing the material eluted from the excized gel pieces on 15% SDS-polyacrylamide gels. The results are shown in Figure 3.

For both cell types we find the four intranucleosomal histones H2A, H2B, H3, and H4 present in equal amounts in each variant. Furthermore, as reported by Varshavsky, et al. (1) and Bakayev, et al. (12) for different cell types, we find a consistent association of H1 (and in the case of erythrocytes, H5) with the lower, but not the higher mobility species. H1 and H5 together are about as abundant in erythrocytes as is H1 alone in liver, suggesting that H5 is associated with a separate subpopulation of low mobility mononucleosomes. A number of nonhistone proteins, possibly of the high mobility group described by Goodwin, Nicolas and Johns (13) are also found with the slower variant. Though the two variants differ in the average lengths of their DNA fragments, this difference cannot account for the discrete and widely separated way in which they migrate on these gels, since there is no size discontinuity

Figure 3. Protein Composition of Low and High Mobility Mononucleosomes. Mononucleosome bands were excized from an ethidium bromide stained gel similar to that in Figure 1. Protein was recovered from the gel slices as described in Materials and Methods. (a) Proteins from low mobility erythrocyte mononucleosomes (CH designates core histones); (b) proteins from high mobility erythrocyte mononucleosomes; (c) proteins from low mobility liver mononucleosomes; (d) proteins from high mobility liver mononucleosomes; (e) proteins from low mobility liver mononucleosomes. Electrophoresis on a 15% SDS gel.

between the two fragment populations. From measurements relative to Hae III digested ϕ X markers we find that the slowly migrating mononucleosomes of liver contain DNA between 206 ± 12 and 164 ± 4 base pairs in length, while the faster migrating population comprises DNA lengths between 159 ± 10 and 138 ± 4 base pairs. For erythrocytes, the corresponding figures are 201 ± 21 and 153 ± 4 base pairs, and 166 ± 9 and 137 ± 3 base pairs, respectively.

Electrophoresis of the Nucleosome Variants in the Presence of Urea

Since the presence of H1 (or H5) appeared to have an exaggerated effect on the electrophoretic mobility of nucleosomes relative to its possible mass or charge contribution, we considered the possibility that the compositional difference in the variants was associated with a conformational difference. High molarity urea has been reported to open up the nucleosome by disrupting the hydrophobic interactions of the carboxy-terminal halves of the intra-nucleosomal histones (14,15) and we reasoned that any conformational difference between the two mononucleosome variants would be minimized in the presence of sufficient urea.

Figure 4 shows the results of electrophoresis of our standard nuclear digests in the presence of 6 M or 8 M urea. Though the particles do not migrate as far into the gel relative to the marker dye as they do in the non-urea gel, a result which is understandable in terms of the extensive shape change induced by the urea treatment (15), it is significant that the nucleosomes migrate as a single peak in this gel system. (This peak contains the full spectrum of mononucleosomes; see below). To underline this point, densitometer scans of the normal and 8 M urea patterns are shown in Fig. 5. The mononucleosome peak on the 8 M urea gels is quite broad; it is nonetheless completely unimodal. The corresponding peak on the 6 M urea gels, also unimodal, is considerably sharper (Fig. 4).

Our determination that the broad lower band of the 8 M urea gels and the somewhat tighter lower band of the 6 M urea gels do in fact contain the mononucleosomes is based on sizing the DNA along the total breadth of these bands, determining the protein content, and examining the electrophoretic mobilities of particles renatured from these bands.

The first test established that the mononucleosomes migrate in rigorous size place with respect to their DNA lengths on the urea gel systems. The appropriate bands from urea gels (Fig. 4) were cut into successive quarters or eighths, the material deproteinized and electrophoresed on a standard low ionic strength gel. Our finding is that the DNA fragments obtained in this way span the gamut of mononucleosome DNA sizes, with decreasing lengths from

Figure 4. Polyacrylamide Gel Electrophoresis of Deoxyribonucleoproteins in the Presence of Urea. Total 2% acid soluble nuclease digests were electrophoresed on 5% gels in the presence of 6 or 8 M urea. (a,b) Erythrocyte digest, 8 M urea, ethidium stain; (c,d) liver digest, 8 M urea, ethidium stain; (e-h) same as (a-d) but stained with Coomassie Blue; (i) erythrocyte digest, no urea; (j) liver digest, 6 M urea, ethidium stain; (k) liver digest, 6 M urea, coomassie blue stain. (Mononucleosomes designated by MN.) Erythrocyte digests in the presence of 6 M urea gave identical pattern to that of liver in 6 M urea (not shown).

the top to the bottom of the urea bands (Fig. 6).

In Fig. 7 proteins obtained from successive quarters of urea mononucleosome bands are displayed on an SDS gel. We established that no protein was lost from the mononucleosomes by passage through the urea gels, by densitometry of identical samples electrophoresed in the presence and absence of urea which were stained with Coomassie Blue. In addition, since nucleosome DNA migrates a similar distance on these urea gels as it does on non-urea gels (our unpublished observations) we could ascertain that no free DNA was generated by this protocol. The relative amounts of the intranucleosomal histones remain unchanged by the urea treatment. The novel element that has been introduced, however, is that H1 (and H5) has redistributed during passage through the urea gel, so that it is now associated with some mononucleosomes of every DNA length in the 140-210 base pair range. This result holds for material run in the presence of 5, 6 and 8 M urea, and for both cell types

Figure 5. Densitometer Scans of Electrophoretic Patterns of Deoxyribonucleo-proteins in the Presence and Absence of Urea. The photographic negatives of the lanes pictured in Figure 4 e and i were scanned. These lanes contained identical amounts of material. (Top) Erythrocyte digest. Electrophoresis in the presence of 8 M urea; (bottom) erythrocyte digest in the absence of urea.

tested. Urea concentrations of 2 M and below leave the mononucleosomes unperturbed (data not shown).

Renaturation of Nucleosomes Presorted According to DNA Size

Re-electrophoresis on a non-urea gel of the intact nucleoprotein particles obtained from successive sevenths or eighths of a urea gel mononucleosome band shows that some nucleosomes with each DNA size now migrate as the low mobility variant. In particular, those with the longest and shortest DNA fragments are found uniformly in this position (Fig. 8). On renaturing, therefore, particles with little and no linker DNA have picked up H1 or H5 and have attained an electrophoretic mobility identical to that of H1 containing particles with linker DNA. This particular gel system, in fact, appears to be insensitive to the added mass and charge of up to 70 base pairs of linker, distinguishing mononucleosomes from one another solely on the basis of their H1 or H5 content. On the other hand, the presence of both H1- (or H5-) containing and H1- (and H5-) lacking nucleosomes in each of the six

Figure 6. Evidence for Redistribution of H1 and H5 in the Presence of Urea. Successive quarters of several mononucleosome bands from an erythrocyte digest electrophoresed on a 6 M urea gel were pooled. Proteins were displayed on a 15% polyacrylamide SDS gel as in Figure 3. (a) Calf thymus histone marker; (b-e) successive quarters from the top to bottom of mononucleosome bands such as that pictured in Fig. 4j. Results with liver were identical to that with erythrocytes, except for the absence of H5. Results with quarters taken from mononucleosome bands from 5 and 8 M urea gels were identical to that obtained with 6 M urea gels (not shown).

intermediate eighths obtained from the urea gels shows that that gel system is insensitive to the H1 content of the unfolded particles, separating nucleosomes from one another solely on the basis of their DNA fragment length.

A corollary of these results is that the nucleosome core particle, which lacks linker DNA, nonetheless has a binding site for H1 or H5.

Renaturation of Nucleosomes of Mixed DNA Size

Since passage through gels containing 5, 6 and 8 M urea caused redistribution of H1 to some nucleosomes of all linker lengths, including no linker, we were interested in the effect of simply pretreating our digests with an effective urea concentration prior to electrophoresis on a non-urea gel. We expected that the urea conditions would still favor the redistribution of H1 to some short DNA nucleosomes, that the electrophoresis would sort the population according to H1 content, and that we would generate a normal bimodal pattern, with the difference that each nucleosome variant would now comprise the full gamut of DNA sizes.

This last conjecture was incorrect. Pretreatment with 5 M urea followed by renaturation of the mixture of nucleosomes without an intervening separation according to DNA size fails to produce any nucleosomes with DNA fragments shorter than 170 base pairs which contain H1 (data not shown). We conclude that either redistribution does not take place in the urea, and that in our previous experiments electrophoresis somehow cooperated with the urea to

Figure 7. DNA Sizes Across Mononucleosome Band from Urea Gel. Successive eighths were excized from an erythrocyte mononucleosome band from an 8 M urea gel, and the DNA was extracted and displayed as in Figure 2. (a-h) DNA from successive eighths (top to bottom) of 8 M urea mononucleosome band; (i,k-p) DNA from successive eighths (top to bottom) of a different 8 M urea mononucleosome band; (j) sample lost before loading on gel; (q) DNA from low mobility erythrocyte mononucleosomes; (r) DNA from high mobility erythrocyte mononucleosomes. Results were identical with liver, and with mononucleosome bands excized from 6 M urea gels (not shown).

effect the transfer of H1, or more likely, redistribution does take place in the urea, but as particles with mixed DNA sizes migrate out of its influence those with 30 base pairs or more of linker compete much more effectively for the H1 molecules. In the size-sorted populations competition does not take place and here the linker-independence of the binding becomes apparent.

Renaturation of Nucleosomes in the Presence of Excess H1 and H5

A confirmation of the urea-dependence of the binding of H1 and H5 by nucleosomes with little and no linker was obtained by incubation of digests in the presence of 100 μ g/ml of a mixture of H1 and H5 (16) followed by electrophoresis on native gels. Only those samples which also contained 5 M urea in the incubation mixture exhibited a shift of all the high mobility material to the low mobility position (not shown). The fact that all of the nucleosomes in our preparation can be made to bind H1 makes us confident

Figure 8. Reelectrophoresis of Urea-Treated Deoxyribonucleoproteins Presorted According to DNA Size. Successive sevenths or eighths of mononucleosome bands were loaded on a 5% non-urea gel and electrophoresed. (a) Total nuclease digest of erythrocyte nuclei; (b-i) successive eighths (top to bottom) of 8 M urea erythrocyte mononucleosome band; (j-p) successive sevenths of 8 M urea liver mononucleosome band; (q) total nuclease digest of liver nuclei. Results were identical with mononucleosome bands excized from 5 and 6 M urea gels (not shown).

that the urea-promoted interaction does not require any linker at all.

In the discussion that follows, "H1" will refer to both H1 and H5, as they behaved similarly in all experiments.

DISCUSSION

Our data show that exposure of nucleosomes to urea can effect the transfer of lysine-rich histones from particles with 30 base pairs or more of linker DNA to particles with little or no linker, and thus confirm, at a more detailed level, a previous report of urea-induced redistribution of H1 in chromatin fragments (17). This redistribution of H1 is quite extensive. Examination of nucleosomes with different DNA fragment lengths which have been re-electrophoresed on non-urea gels (Fig. 8) show an abundance of slow

migrating (H1-containing) material among the linkerless core particles. Moreover, the pattern of distribution of H1 we find across the broad mononucleosome bands on urea gels (Fig. 7) shows comparable amounts of this protein among particles with all DNA sizes. These results lead us to believe that urea treatment exposes a site on the nucleosome to which the H1 molecule can bind in the absence of linker. The binding must be quite strong: unbound H1 would migrate in the opposite direction from nucleosomes on our electrophoretic gels. Furthermore, it is likely to be an ionic interaction with the DNA, as it is not disrupted in the presence of urea. One possibility that suggests itself is that the conformation of the core particle is significantly different in the H1-containing and H1-lacking nucleosomes, and that the H1 is somehow trapped by an interaction with the DNA that requires a structural rearrangement. An alternative explanation of the urea effect might be that H1 itself undergoes a conformational change which stimulates its binding to the nucleosome. In either case the protein might actually bind to some "pseudo-linker" released by the slippage of DNA off the core particle. Though we do not exclude any of these possibilities, the idea of a conformational change in the core particle stabilized by H1 is consistent with certain other evidence.

It is striking that the electrophoretic mobility of H1-containing particles is virtually identical on the non-urea gels regardless of how much linker is present. The same holds for the distinctly different mobility of H1-lacking particles (Fig. 8). Though we do not know in detail what function of charge, mass and conformation determines the mobility of nucleoprotein particles on these gels, it is clear that it is very insensitive to added charge and mass in the form of linker DNA. The fact that H1 causes a similar mobility shift across the gamut of nucleosome sizes suggests that it is interacting similarly with them all, i.e. primarily at the core site. The nucleosomes which have been re-electrophoresed subsequent to urea treatment can be considered "native" by the criteria of composition and of electrophoretic mobility, a more sensitive assay in the case of these particles than sedimentation rate (1). We have made no attempt to determine how the relation of DNA to protein may have been altered by our protocol, as reflected in the distribution of DNAase I susceptible sites, for example (18).

Since the presence of urea during electrophoresis simultaneously sorts mononucleosomes according to DNA size and allows H1 to associate with a subset of particles of every DNA length, we can ask the following question: how

would a population of nucleosomes containing a given length of DNA, some with H1 and some without, electrophorese in an environment where conformational differences are minimized? The answer, obtained from a comparison of Figs. 4 and 6, is that they migrate identically. That is, a given thin slice of the urea mononucleosome band contains H1-containing and H1-lacking particles of identical DNA size. The presence of urea could be equalizing the conformations of particles which are conformationally different in the native state, or alternatively, it could be equalizing the shapes of particles with similar conformations but different frictional coefficients resulting from the presence or absence of H1. We favor the former interpretation because the urea dependence of the H1 redistribution suggests (though it does not prove) that the H1-containing and H1-lacking particles are conformationally different. In addition, preliminary experiments show that H1-containing and H1-depleted nucleosomes are differentially susceptible to the crosslinking agent dithiobis (succinimidyl propionate) (our unpublished results). We therefore tentatively suggest that H1 interacts with a site at the nucleosome core to induce or stabilize a conformational change.

Finally, studies in several laboratories have shown that transcriptionally activated nucleosomes are conformationally different from bulk nucleosomes (8,19). In a separate report one of us has proposed a role for H1 in stabilizing the template inactive conformation (20). We are therefore investigating the relation between possible nucleosome conformational heterogeneity related to H1 content and that related to genomic function.

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