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On the Mechanism of Nucleosome Unfolding†

Harold G. Martinson* and Rod J. True

ABSTRACT: We have studied the relative stabilities to urea denaturation of histone–histone binding interactions as they occur both in chromatin and in histone complexes free in solution. We have used the two zero-length contact-site cross-linking agents, tetranitromethane and UV light, to measure the relative degree of H2B–H4 and H2A–H2B association under various conditions. The two interactions were disrupted coordinately when nuclei were treated with increasing concentrations of urea. In contrast, when histone complexes in 2 M NaCl were treated with urea, the H2B–H4 interaction was found to be much less stable than the H2A–H2B interaction. We have shown previously that nucleosomes unfold at low ionic strengths such that the H2B–H4 but not the H2A–H2B interaction is broken in the process. We speculate that the preferential rupture of the H2B–H4 contact is of physiological significance.

In eukaryotes, chromosomal DNA is packaged tightly into small subunits called nucleosomes (Kornberg, 1977; Felsenfeld, 1978). Nucleosomes consist of a compact histone core composed of two each of histones 2A, 2B, 3, and 4 about which are wrapped nearly two turns of DNA 144 base pairs long. A fundamental problem which arises in considering the genetic functions of DNA in chromatin concerns the mechanism by which DNA packaged so tightly can be utilized as a template in replication and transcription. Presumably the nucleosome must unfold in some way to facilitate these functions.

In the preceding paper (Martinson et al., 1979a), we presented studies directed at characterizing the mode of nucleosome unfolding in vitro in response to low ionic strength. Those studies were done in the absence of denaturants and may therefore reflect conformational transitions which occur in vivo. We found that nucleosome unfolding resulted in the disruption of one major histone–histone binding interaction within the nucleosome core (H2B–H4) but that another of the major interactions (H2A–H2B) was unaffected (Martinson et al., 1979a). This result would not have been predicted based on the equivalent pairwise affinities of these histones for each other in solution (Van Holde & Isenberg, 1975). However, it is not surprising that the complex interrelationships of components within the native nucleosome should influence the binding parameters of individual histone pairs. We therefore decided to investigate the effects of various parameters on the relative stabilities of the H2B–H4 and H2A–H2B interactions as they occur in complex systems such as chromatin or complete mixtures of the histones.

We have approached this problem by studying the cross-linking patterns of histones and chromatin as a function of salt and urea concentrations, using the highly specific binding site cross-linkers, tetranitromethane [$C(NO_2)_4$] and UV light (Martinson & McCarthy, 1975, 1976; Martinson et al., 1976). Numerous studies on the destabilization of chromatin and nucleosomes by salt and urea have been carried out (e.g., Whitlock & Simpson, 1976; Carlson et al., 1975; Olins et al., 1977; Weintraub & Van Lente, 1974; Chang & Li, 1974; Hardison et al., 1977). However, these studies do not provide information on specific histone–histone interactions. The predominant cross-linked product induced by $C(NO_2)_4$ in nuclei is an H2B–H4 dimer. The main product induced by UV irradiation is an H2A–H2B dimer. In addition, UV, like $C(NO_2)_4$, induces the specific formation of an H2B–H4 dimer in which the cross-link, however, is at a slightly different position from the one in the homologous $C(NO_2)_4$ dimer (Martinson et al., 1979b). The use of highly selective probes of this type permits data on specific histone–histone binding interactions to be obtained directly in complex systems and obviates the need for prior purification of individual components.

Our results show that urea-induced denaturation of the nucleosome does not follow a sequential pathway involving specific initial rupture of the H2B–H4 contact as we had expected based on our results on unfolding in low salt (Martinson et al., 1979a). Instead we find that the H2B–H4 and H2A–H2B binding interactions in nucleosomes are denatured by urea coordinately. However, if the histone core in 2 M salt (free of DNA) is denatured by urea, a coordinate effect is no longer observed, and the H2B–H4 interaction is found now to be considerably less stable than the H2A–H2B interaction. We speculate that the H2B–H4 interaction is

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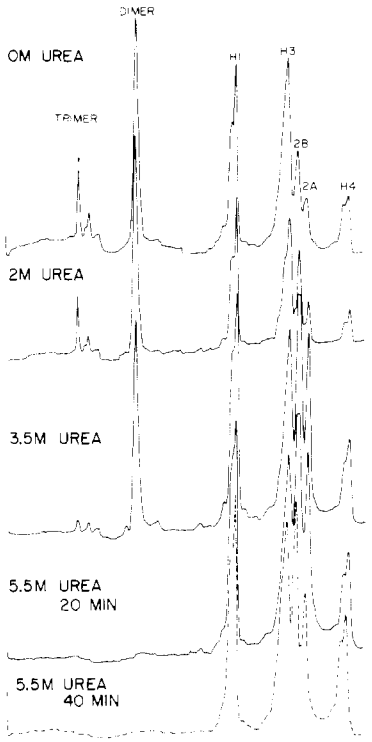

FIGURE 1: UV cross-linking of nuclei in urea. Nuclei were suspended in phosphate-buffered saline–EDTA at an A_{260} of ~50. They were then carefully diluted with nine volumes of phosphate-buffered saline–EDTA containing various concentrations of urea such that the final concentrations were as indicated in the figure. Vigorous mixing was avoided as this gave rise to extensive foaming and intractable aggregates. A certain amount of aggregation inevitably occurred upon standing for a few minutes, however. Samples (2 mL) were irradiated in 9-mm internal diameter quartz tubes for 20 (—) or 40 min (- - -). After photolysis, all of the samples were dialzyed against 0.4 N H_2SO_4 overnight in the cold. The dialysates were centrifuged and the histones collected by acetone precipitation as usual.

designed to facilitate nucleosome unfolding in vivo in response to mechanical, electrostatic, or other physiological (nondenaturing) stresses.

Materials and Methods

Calf-thymus nuclei were prepared from thymus homogenized in phosphate-buffered saline–EDTA as previously described (Martinson et al., 1979a). UV irradiation in small quartz tubes was also done as before. $C(NO_2)_4$ cross-linking was done as before except that small samples rather than 1-L batches were used (Martinson et al., 1979a). Our standard procedure was to add a constant 5 μL of pure $C(NO_2)_4$ to all volumes (0.5–2 mL) of the nuclei, chromatin, or histone suspensions since in large excess, as this is, the actual amount of $C(NO_2)_4$ is relatively unimportant. All polyacrylamide gel electrophoreses were done on acid–urea (Panyim & Chalkley, 1969) gels which were stained with Coomassie Blue and destained with acetic acid as described previously (Martinson & McCarthy, 1975). The gels were scanned with a Joyce Loebl densitometer. In all the figures, migration is from left to right with the cross-linked components appearing in the leftward 50% of each scan. Histones were prepared from nuclei or chromatin by extraction in the cold with sulfuric acid and precipitation with acetone.

Results

Unfolding of Nuclei by Urea

H2A–H2B Contact. When nuclei suspended in phosphate–buffered saline–EDTA are treated with increasing

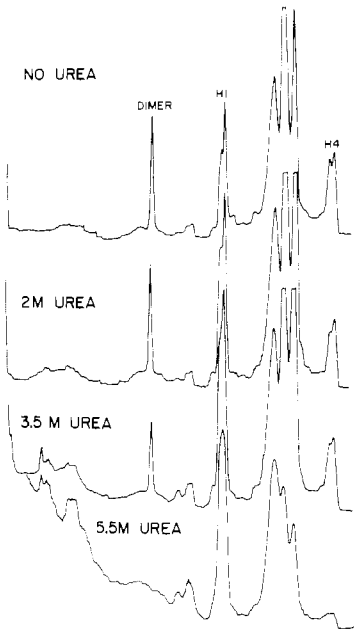

FIGURE 2: $C(NO_2)_4$ cross-linking of nuclei in urea. Samples were prepared exactly as described in the legend to Figure 1 and then cross-linked with $C(NO_2)_4$ for 15 min in the presence of urea at the concentrations indicated in the figure. The cross-linking reaction was stopped by the addition of H_2SO_4 to 0.4 N, and the samples were then dialyzed free of urea and the histones prepared for analysis as described for Figure 1.

concentrations of urea, the H2A–H2B binding interaction becomes ruptured at urea concentrations between 4 and 5 M. This is illustrated in Figure 1, which shows that nuclei suspended in 3.5 M urea give good yields of H2A–H2B dimer upon irradiation with UV light, whereas nuclei suspended in 5.5 M urea do not yield a dimer. Even twice the normal dose of UV fails to yield any detectable dimer despite the fact that considerable acid-extractable monomer remains (Figure 1, bottom profile). This is not a chemical effect of urea on cross-linking, since histones free in solution are readily cross-linked by UV in the presence of 5.5 M urea to yield an H2A–H2B dimer (see below).

H2B–H4 Contact. The H2B–H4 interaction of nuclei also is disrupted by urea at concentrations between 4 and 5 M. This is shown in Figure 2 where it is seen that $C(NO_2)_4$ cross-linking gives rise to the usual H2B–H4 dimer in 3.5 M urea but not in 5.5 M urea.

The presence of a large amount of slowly migrating material in the bottom scan of Figure 2 leaves open the possibility that in the presence of urea, $C(NO_2)_4$ does induce the specific formation of the H2B–H4 dimer but that these dimers then rapidly enter into the formation of higher aggregates. We consider this to be unlikely for two reasons. First, in other experiments (not shown), we have determined that cross-linking for four times as long in 3.5 M urea also builds up a considerable background of low-mobility material in the gel. Nevertheless, the dimer is still visible above the background, and, although there is a general histone loss, there is no evidence of preferential loss of dimer compared to the histone monomers. Secondly, nuclei can be cross-linked overnight in the absence of urea with vast excesses of $C(NO_2)_4$ being added during the course of the reaction; yet there is negligible loss of monomeric and dimeric histones, and little background appears. Thus the high background induced by $C(NO_2)_4$ in the presence of 5.5 M urea apparently reflects extensive nonspecific cross-linking arising from collisions of the denatured histones bound along the DNA. If a significant

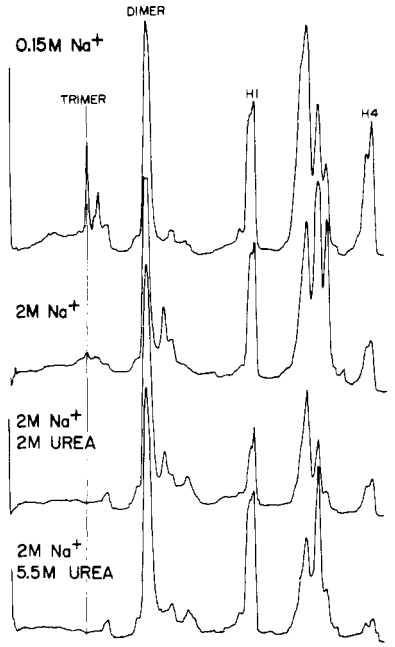

FIGURE 3: UV cross-linking of nuclei suspended in salt and urea. This experiment was conducted as described for Figure 1, except that the nuclei were diluted into solutions, giving the additional Na^+ or urea concentrations indicated. (Note that 2 M additional $[Na^+] \equiv 2.15$ M final $[Na^+]$.) The irradiation time was 20 min for each sample. The results do not depend on the order of addition of the salt and urea.

amount of H2B–H4 cross-linking does occur in this context, it would be as part of a larger nonspecific process rather than as a result of specific H2B–H4 interactions.

The effect of urea on H2B–H4 cross-linking by UV confirms this interpretation. UV induces the formation of both H2A–H2B and H2B–H4 dimers which comigrate on acid–urea gels (Martinson et al., 1979a,b). The H2A–H2B dimer is produced in much higher yield than the H2B–H4 dimer and therefore dominates the cross-linking pattern at urea concentrations of 3.5 M and below (see Figure 1). However, the absence of *any* dimer in 5.5 M urea demonstrates that neither the H2A–H2B nor the H2B–H4 cross-link is formed at this urea concentration (Figure 1).

It may be noted that the formation of the UV-induced H2A–H2B–H4 trimer (Martinson et al., 1979b) of Figure 1 also is abolished in 5.5 M urea. In addition, the reduction in yield of this trimer is more dramatic between 2 and 3.5 M urea than for either of its dimer precursors discussed above. This is to be expected, since both of the trimer's component contact sites are destabilized slightly in that range.

The finding of no obvious difference in the stabilities to urea denaturation of the H2A–H2B and H2B–H4 binding interactions in chromatin was unexpected in view of our previous finding that exposure to very low ionic strength resulted in complete disruption of the H2B–H4 contact while leaving the H2A–H2B contact unaffected. We therefore sought to characterize the relative stabilities of these two binding interactions in complexes of the four core histones free of DNA in 2 M salt.

Intrinsic Stability of the Histone Core

Disruption of Nuclei in High Salt Concentration. From the top two scans of Figures 3 and 4, it can be seen that liberating the histones from DNA by use of 2 M Na^+ disrupts neither the H2A–H2B (Figure 3) nor the H2B–H4 (Figure 4) contact, although the efficiency of specific cross-linking appears to be a little lower than for intact nuclei. These results

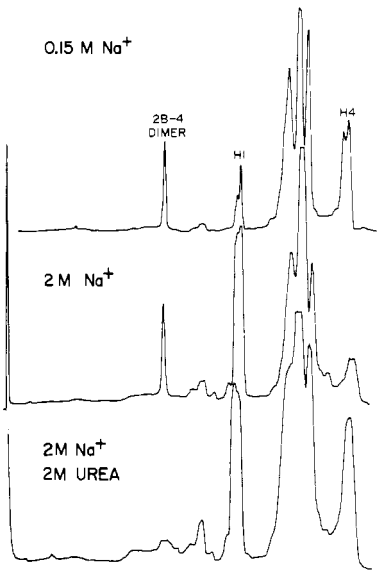

FIGURE 4: $C(NO_2)_4$ cross-linking of nuclei suspended in salt and urea. This experiment was done exactly as described for Figure 2, except that cross-linking was conducted in solutions containing the additional concentrations of Na^+ and urea indicated in the figure. The results do not depend on the order of addition of the salt and urea.

are consistent with the reports of others (Weintraub et al., 1975; Thomas & Butler, 1977; Thomas et al., 1977; Lilley et al., 1977) that histone–histone interactions are not greatly perturbed by the removal of DNA with salt. However, the histones are no longer constrained by the DNA so it is noteworthy in Figure 3 that even the rudiments of the characteristic trimer pattern persist after UV cross-linking in 2 M salt.

Reformation of Histone–Histone Contacts in the Absence of DNA. Weintraub et al. (1975) have suggested that even in 2 M salt DNA may have some organizing influence on the histones. In order to determine whether the histones alone possess sufficient information for proper folding according to our cross-linking criteria, we renatured acid-extracted histones dissolved in urea by the addition of 2.8 M NaCl. The resulting histones in 2.5 M NaCl gave UV and $C(NO_2)_4$ cross-linking patterns very similar to those of intact nuclei (Figure 5) and almost indistinguishable from those of nuclei suspended in 2 M salt. We thus conclude that the histones alone contain the information for folding which gives rise to the specific H2A–H2B and H2B–H4 contacts measured by UV and $C(NO_2)_4$ cross-linking. This extends the finding of Weintraub et al. (1975) that acid-extracted histones in 2 M salt yield a trypsin-resistant core like that of native chromatin and confirms that acid-extracted histones can be "renatured" (see Bidney & Reeck, 1977).

The persistence of the histone core structure, free of DNA in 2 M salt, makes it possible to explore the intrinsic stabilities of the H2A–H2B and H2B–H4 interactions as they occur in histone cores free of any additional constraints (i.e., those imposed by DNA).

Relative Stabilities of Histone–Histone Interactions in 2 M Salt. The lower scans in Figures 3 and 4 show that the H2B–H4 contact is much less stable to urea than the H2A–H2B contact for histone complexes in 2 M salt. Figure 3 shows that the H2A–H2B interaction is very stable to urea and that, in the presence of 2 M salt, urea concentrations as high as 5.5 M do not significantly affect dimer production by UV irradiation. In contrast, the bottom panel of Figure 4 shows that even 2 M urea is sufficient to disrupt the H2B–H4 interaction responsible for $C(NO_2)_4$ cross-linking. This is *not*

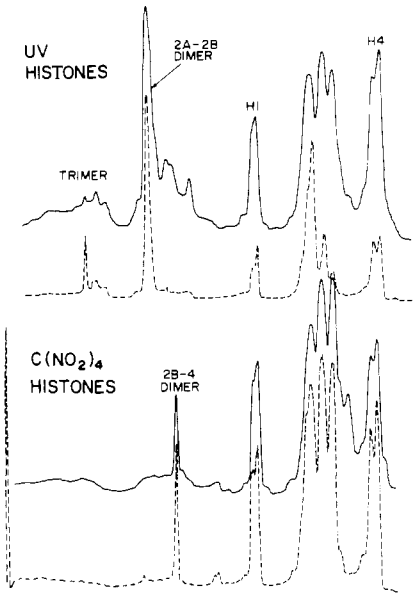

FIGURE 5: Refolding of acid-extracted histones in the absence of DNA. The top tracing of the figure is of UV-cross-linking acid-extracted histones in 2.5 M NaCl. The dashed tracing just below it is from histones of nuclei cross-linked at physiological ionic strength and is the same sample as that represented by the dashed line in Figure 2 of Martinson et al. (1979a). The lower two tracings in the figure are of $C(NO_2)_4$-cross-linked material, the solid tracing being cross-linked histones in 2.5 M NaCl and the dashed tracing being of histones extracted from cross-linked nuclei. The latter is the same sample as that depicted in the top scan of Figure 5 of Martinson et al. (1979a). Histones were acid-extracted as described (Martinson et al., 1979a), except that the nuclei were washed twice with 0.35 M NaCl prior to extraction. The acetone-precipitated histone sulfates were dissolved in water at a concentration of 12–13 mg/mL. To 0.5 mL of this solution were added 0.3 g of urea and 7 μL of 1 N HCl, giving about 7 M urea and 10 mM HCl under which conditions aggregation is expected to be eliminated. Seven milliliters of 2.8 M NaCl, 50 mM sodium phosphate, pH 7.5, was then added very slowly with mixing, giving about 2.5 M NaCl, 0.6–0.7 M urea, and 0.8 mg/mL of histone. The residual urea seems to be helpful in reducing aggregation and nonspecific cross-linking. UV irradiation was carried out on an aliquot of this histone mixture for 1 min in a 7-mm internal diameter quartz tube. The irradiation dose is much less than usual because of the absence of DNA which absorbs strongly in the UV, thus shielding the histones. Although the stated irradiation times for chromatin usually include lamp warm-up time, for histones, irradiation was at full lamp intensity throughout. $C(NO_2)_4$ cross-linking of the histones was carried out as usual on a 1-mL aliquot for 20 min.

a chemical effect of urea on the $C(NO_2)_4$ reaction since, as shown in Figure 2, even 3.5 M urea does not have a large effect on $C(NO_2)_4$ cross-linking of chromatin. Moreover, Figure 3 provides independent evidence that the H2B–H4 contact is considerably less stable than the H2A–H2B contact. Figure 3 shows that, whereas even 5.5 M urea has little effect on production of the H2A–H2B dimer, 2 M urea completely abolishes formation of the H2A–H2B–H4 trimer, presumably by disrupting its constituent H2B–H4 contact. This in turn is not a chemical effect of urea, since the trimer *is* formed in chromatin in the presence of even 3.5 M urea (Figure 1). It thus is apparent that in 2 M salt the H2A–H2B interaction is much more stable to urea than the H2B–H4 interaction.

Role of DNA in Nucleosome Structure. A comparison of the results obtained from nuclei (Figures 1 and 2) with those from histones solubilized in 2 M salt (Figures 3 and 4) shows that, although the H2B–H4 interaction is intrinsically much less stable to urea than the H2A–H2B interaction (i.e., in 2 M salt), the two interactions are nevertheless of similar stability in chromatin (i.e., in 0.15 M salt). This contrast is highlighted by the summary presented in Table I.

Table I: Presence (+) or Absence (–) of Histone–Histone Contacts in Nuclei as a Function of Urea and Salt Concentrations

histone–histone interaction	urea concn (M)	salt concn 0.15 M	salt concn 2.15 M
H2A–H2B	2–3.5	+	+
	5.5	–	+
H2B–H4	2–3.5	+	–
	5.5	–	–

A salt concentration of approximately 2 M (actually 2.15 M) was chosen for our studies because histones in 2 M salt are actually more stable than histones in nucleosomes to overall urea denaturation as measured by circular dichroism (Olins et al., 1977). Thus the markedly reduced stability to urea of the H2B–H4 contact of free histone complexes in 2 M salt, compared to the histone cores in chromatin at 0.15 M salt, is clearly significant (see Table I). On the other hand, we cannot be sure of the significance of the enhanced stability of the H2A–H2B interaction in 2 M salt as compared to that in chromatin (see Table I). This observation could reflect a more effective charge shielding by the salt at that concentration than by DNA. It could also reflect steric strain imposed by the coiled DNA on the H2A–H2B complex. The latter factor would imply a role of H2A and H2B in coiling DNA as suggested by Levin et al. (1978) and Klevan et al. (1978).

Discussion

Heterotypic → Homotypic Disproportionation of Histone Complexes. Using specific cross-linking as a measure of histone–histone binding, we have studied the effects of urea on the stabilities of the H2A–H2B and H2B–H4 interactions. The cross-linking agents used, UV light and $C(NO_2)_4$, are highly specific, zero-length cross-linkers which probably induce their cross-links within the actual histone–histone binding sites (Martinson et al., 1976; Martinson & McCarthy, 1975, 1976). These cross-linking agents are therefore well suited to the study of specific histone–histone interactions within a complex mixture. Although such cross-linking reactions suffer from problems of quantitation, they are, nevertheless, superior to most physical techniques with respect to specificity.

Employing this specific cross-linking approach, we have confirmed that histones, either removed directly from chromatin with 2 M salt or else renatured from acid-extracted material into 2 M salt, form heterotypic complexes (Weintraub et al., 1975; Thomas & Butler, 1977). Weintraub et al. (1975) have defined heterotypic complexes as those which contain a mixture of arginine-rich and slightly lysine-rich histones. Homotypic complexes are then those which involve either only the arginine-rich histones (H3 and H4) or only the slightly lysine-rich histones (H2B and H2A).

Our studies on the heterotypic complex in 2 M NaCl have shown that the H2A–H2B interaction is very stable to urea denaturation at this salt concentration—even more stable than in nuclei at physiological ionic strength. In contrast, the H2B–H4 interaction of the heterotypic complex is easily disrupted by urea, being much less stable than in nuclei or than the H2A–H2B interaction.

These results are not at variance with the reports that the H2B–H4 and H2A–H2B interactions display similar stabilities when the histones are combined pairwise in dilute buffer (D'Anna & Isenberg, 1973, 1974). The methods and conditions of measurement are not comparable, and our experiments were conducted on total unfractionated histone in which, for example, the interaction of H3 with H4 may decrease the affinity of H4 for H2B.

The observation that the H2B–H2A interaction persists in preference to the H2B–H4 interaction under certain conditions is not new of course and was one feature incorporated into the original subunit model of Kornberg (1974). Weintraub et al. (1975) have since emphasized the heterotypic → homotypic nature of this disproportionation reaction. Both of these groups studied disproportionation in the absence of denaturants, showing that the reaction could be induced by changes in pH, salt concentration, or even histone concentration. We have repeated some of these studies, using specific cross-linking with comparable results (unpublished experiments). Our present studies now add urea to the list of variables which can induce this response and provide further evidence that the disproportionation reaction is a fundamental capability of the heterotypic complex.

It should be noted that while our results are similar to those of the other groups, the techniques used differ considerably. Thus both of these groups studied disproportionation by histone fractionation procedures, whereas we have studied the reaction in the constant presence of total histone by assaying for the intactness of individual histone–histone binding sites [for a justification of the use of $C(NO_2)_4$ and UV light as binding site probes, see Martinson & McCarthy (1975, 1976), Martinson et al. (1976), and DeLange et al. (1979)].

Olins et al. (1977) have recently reported circular dichroism studies which show that urea denaturation of the histone core in 2 M salt is cooperative. Our results showing that in 2 M salt the H2B–H4 and H2A–H2B interactions are disrupted separately are not in disagreement with theirs since, as they have pointed out, circular dichroism is responsive primarily to denaturation of the homotypic complexes and is not very sensitive to disproportionation alone. Thus our data, in combination with those of Olins et al. (1977), show that disproportionation precedes overall denaturation of histones in 2 M salt. Moreover, their finding of a sharp overall denaturation transition induced by urea suggests that the separated homotypic complexes (H2A–H2B as well as H3–H4) denature simultaneously. We have found (unpublished experiments) that for free histones the dependence of H2A–H2B cross-linking on salt and urea concentrations is similar to that measured for total histone by Olins et al. (1977) using circular dichroism. We therefore infer that disruption of the specific homotypic interactions is part of the final overall histone denaturation process.

Mechanism of Nucleosome Unfolding. Although the heterotypic complex in 2 M salt undergoes disproportionation prior to overall denaturation as urea is added, our results show that within chromatin this is not the response of histones to urea. The H2B–H4 interaction, which is very labile at low urea concentrations in 2 M salt, is as stable as the H2A–H2B interaction when studied in chromatin. Indeed, urea denaturation of chromatin at physiological salt concentration gives rise to coordinate rupture of the H2B–H4 and H2A–H2B interactions. This is in obvious contrast to the manner in which chromatin unfolds in response to low ionic strength (Martinson et al., 1979a).

The response of chromatin to low ionic strength is much like the response of heterotypic histone complexes in 2 M salt to intermediate concentrations of urea, namely, the H2B–H4 but not the H2A–H2B interaction is ruptured. This suggests that nucleosome unfolding at low ionic strength reflects an innate tendency of the histone core to dissociate in a particular way.

Since the H2B–H4 interaction is preferentially disrupted in chromatin by low ionic strength and in histone complexes by urea, why is it not preferentially disrupted in the case of the chromatin–urea combination? Apparently the H2B–H4 interaction is not significantly weakened by the urea prior to the onset of general histone denaturation for, if it were, the nucleosome would be expected to initiate unfolding at that site much as occurs at low ionic strength. Perhaps the more stable H2A–H2B and H3–H4 binding interactions maintain nucleosome integrity in such a way that at moderate urea concentrations urea is excluded from the H2B–H4 binding site. Thus the nucleosome does not begin to unfold until sufficient urea is added to denature the stable interactions themselves. Such an exclusion hypothesis would account for the difference between chemically induced denaturation (urea) and physically induced unfolding (low ionic strength). Thus the electrostatic stress of low ionic strength could induce the preferential rupture of the intrinsically weaker H2B–H4 interaction regardless of whether that binding site were accessible to the solvent. The exclusion hypothesis would also be consistent with the urea denaturation results for the heterotypic complex, since in 2 M salt this complex is known to have a more open structure than the nucleosome (Olins et al., 1977; Zama et al., 1977). The exclusion proposal is not inconsistent with the fact that the H2B–H4 binding site can be cross-linked, since both UV and $C(NO_2)_4$ can penetrate to the hydrophobic interior of proteins (Martinson & McCarthy, 1975; Martinson et al., 1976).

It seems unlikely that the pathway of urea denaturation of chromatin will serve as a useful model for physiological conformational changes. On the other hand, the more limited and orderly unfolding of the nucleosome which occurs at low salt concentrations (Martinson et al., 1979a) may well reflect the kinds of changes which occur in vivo. Important localized changes in electrostatic effects are brought on in vivo by covalent histone modifications and by association of various nonhistones with the nucleosome. Furthermore, obvious mechanical stresses accompany replication and transcription. Thus the electrostatic stress of low ionic strength resembles the kinds of stress likely to be of importance in vivo.

The results presented above complement those of Bina-Stein & Simpson (1977), Moss et al. (1977), and Camerini-Otero & Felsenfeld (1977) who have documented the central role of H3 and H4 in nucleosome structure. Our present results show that the H2A–H2B interaction of the histone core is considerably more stable to urea than is the H2B–H4 interaction. Our previous results have shown further that the H2A–H2B interaction remains intact while the H2B–H4 interaction becomes ruptured as chromatin is extended in distilled water. It therefore seems likely that the H3–H4 and H2A–H2B interactions together constitute the fundamental matrix of nucleosome structure. We will call these interactions *skeletal* interactions and suggest that they are invariant in vivo, providing a structural framework which is the basis not only for the usual nucleosome structure itself but also for the various alternate conformational states which the nucleosome can assume.

In undergoing conformational transitions from one state to another, certain histone–histone interactions must obviously change. We will call these the *modal* interactions. Prominent among these must be the interaction between H2B and H4. The H2B–H4 interaction is an extensive one in which particular contact points between H2B and H4 can vary, depending on the conformational state of the nucleosome (Martinson et al., 1979a). Camerini-Otero & Felsenfeld (1977) have provided calculations which show that a single histone–histone binding interaction could reasonably be expected to provide the energetic difference between a folded

and an unfolded nucleosome. The H2B–H4 interaction could well provide this pivotal influence as a modal contact.

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Isolation and Identification of 24,25-Dihydroxyvitamin D_2 Using the Perfused Rat Kidney†

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ABSTRACT: 24,25-Dihydroxyvitamin D_2 was biologically generated from synthetic 25-hydroxyvitamin D_2 using an isolated perfused rat kidney incubated under normocalcemic and normophosphatemic conditions. 24(*R*),25-Dihydroxyvitamin D_2 and 24(*S*),25-dihydroxyvitamin D_2 were chemically synthesized starting with stigmasterol and their configurations determined by X-ray diffraction analysis. The biosynthetic metabolite proved to be identical with the synthetic 24(*R*) epimer in its chromatographic mobility, mass spectrometry, and derivative synthesis. Significant quantities of [3α-^{3}H]-24(*R*),25-dihydroxyvitamin D_2 were found to be present in the plasma of vitamin D replete rats 24 h after receiving a physiological dose of [3α-^{3}H]vitamin D_2.

24,25-Dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$)[1] is a major product of renal 25-OH-D_3 metabolism (Suda et al., 1970; Holick et al., 1972) in normocalcemic and normophosphatemic animals receiving adequate intakes of vitamin D (Boyle et al., 1971; Knutson & DeLuca, 1974; Friedlander et al., 1977). Chemical synthesis of the two possible epimers of 24,25-$(OH)_2D_3$ (24(*R*)-hydroxy and 24(*S*)-hydroxy) has led to identification of the natural metabolite with a 24(*R*) configuration (Ikekawa et al., 1977). The role of 24(*R*),25-$(OH)_2D_3$ remains obscure, although its rapid excretion in the

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[1] Abbreviations used: 24,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; 24,25-$(OH)_2D_2$; 24,25-dihydroxyvitamin D_2; 24-OH-D_2, 24-hydroxyvitamin D_2; 25-OH-D_3, 25-hydroxyvitamin D_3; 25-OH-D_2, 25-hydroxyvitamin D_2; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 1,25-$(OH)_2D_2$, 1,25-dihydroxyvitamin D_2; LC, high performance liquid chromatography; PTH, parathyroid hormone.

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