Differential Dissociation of Histone Tails from Core Chromatin†

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ABSTRACT: The dissociation of the trypsin-sensitive basic tails of the core histones in core chromatin has been followed as a function of [NaCl] using proton NMR spectroscopy. The tails dissociate in a highly cooperative all or none manner over the salt concentration range 0.2–0.6 M, that is, below the salt concentration required to dissociate the complete molecule. Assuming that each basic tail dissociates independently, the total number of salt linkages involved in binding the tails to DNA is 103. This equals the number of basic side chains in the tails of an octamer. The standard free energy of dissociation, $\Delta G°$, in 1 M NaCl at 297 K is 3.6 kcal/mol. Temperature had no effect on the extent of dissociation up to 45 °C. However, between 45 and 65 °C, where the premelting transition in the core chromatin occurs, the tails dissociated completely. Dissociation of the tails was associated with a conformational transition in the DNA consistent with loss of supercoiling. From this, and the results of a previous study, it can be shown that the structured, trypsin-resistant domain of each core histone octamer makes 100 salt linkages to DNA. Thus, in 10 mM salt, each core octamer makes a total of 203 salt linkages to DNA.

The binding of the core histones to DNA is dominated by electrostatic interactions involving salt linkages between the DNA phosphates and positive charges on the histone side chains. An understanding of the structure in terms of the forces holding the complex together will therefore require a knowledge of the number, strength, and disposition of these salt linkages. A recent thermodynamic analysis of the complete dissociation of the core histones from DNA has shown that there are about 28 salt links formed between each H3–H4 tetramer and DNA and about 30 salt links between each H2A–H2B dimer and DNA. In addition, the large positive enthalpy of binding both the arginine-rich and the lysine-rich histones implies that there are also significant nonelectrostatic contributions to the binding possibly involving hydrophobic interactions (Burton et al., 1978). In this previous analysis, it was not possible to determine which basic side chains on the histone primary sequences were involved in the salt links, and it was assumed that they were mainly in the basic amino- and carboxyl-terminal tails. However, it has been shown that in core particles (Whitlock & Stein, 1978) and core chromatin (Diaz & Walker, 1983) the basic tails can be degraded by trypsin to leave a relatively compact histone complex still bound to DNA. Furthermore, in core particles, the majority of the side chains in the basic regions of H2A and H2B are not bound to DNA, and those of H3 and H4 that are bound appear to be released in 0.6 M NaCl before complete dissociation of the histones occurs (Cary et al., 1978). These results imply that the major sites of interaction between DNA and histone in both core particles and core chromatin are not in the basic tails, which appear to bind relatively weakly, but in the structured domain of each histone. Here the dissociation of the basic tails from the core chromatin complex is investigated in some detail as a function of NaCl concentration. It is shown that the tails of both the lysine-rich and the arginine-rich histones are bound at low salt but become completely dissociated at salt concentrations below those required to dissociate the histones completely.

Proton NMR spectroscopy has been used to follow the dissociation. It has been well established that in the H2A–H2B dimer (Moss et al., 1976a), the H3–H4 tetramer (Moss et al., 1976b), and the core histone octamer (Cary et al., 1978) the basic tails are in a relatively mobile conformation and the proton NMR spectrum is characterized by sharp spectral lines arising from residues in the tails. These sharp spectral lines are not present in chromatin (Bradbury et al., 1973; Davies & Walker, 1974) or in H1-depleted chromatin in low salt (Diaz & Walker, 1983). In both these complexes, therefore, the tails appear to be tightly bound to the DNA. The release of the tails from the DNA with increasing salt concentration or temperature can therefore be followed by the progressive development of the NMR spectrum.

Experimental Procedures

Chicken erythrocyte chromatin depleted of histones H1 and H5 (referred to as core chromatin) was prepared as described previously (Diaz & Walker, 1983). The core chromatin was dialyzed exhaustively against D_2O containing sodium dihydrogen phosphate and disodium hydrogen phosphate (1 mM), pH 7.0. The NaCl concentration was adjusted by adding a concentrated solution of NaCl in D_2O buffered as above or by dialysis into NaCl. Identical results were obtained from both methods. Core histone was prepared by extraction of core chromatin with 0.3 N HCl. The precipitate of DNA was removed by centrifugation and the solution of core histones dialyzed against 2 M NaCl and 10 mM sodium phosphate, pH 7.0, by using the protocol described by Beaudette et al. (1981). The concentration of the core histone was determined by using an extinction coefficient at 276 nm of 0.43 mg mL^{-1} cm^{-1} (Ruiz-Carillo & Jorcano, 1979).

Proton NMR spectroscopy was carried out by using a Bruker 300 spectrometer operating routinely at 297 K in the Fourier-transform mode. Chemical shifts were measured relative to sodium 3-(trimethylsilyl)propionate-d_4 (TSP). Spectral assignments were made as described previously (Diaz & Walker, 1983). An analysis of the ^1H spectrum of N^ϵ-methyllysine showed that the N-methyl protons could be assigned to a resonance at 2.7 ppm. On the basis of this, the small peak at 2.8 ppm in the chromatin spectrum is tentatively assigned to the methyl protons of N^ϵ-methyllysine. The areas of absorption peaks in the NMR spectra were determined by integration and expressed relative to the area of an internal standard of TSP which was included in all samples, usually at a concentration of 1 mM. The number of protons contributing to a particular resonance was then converted directly to a concentration from the amino acid composition. The use

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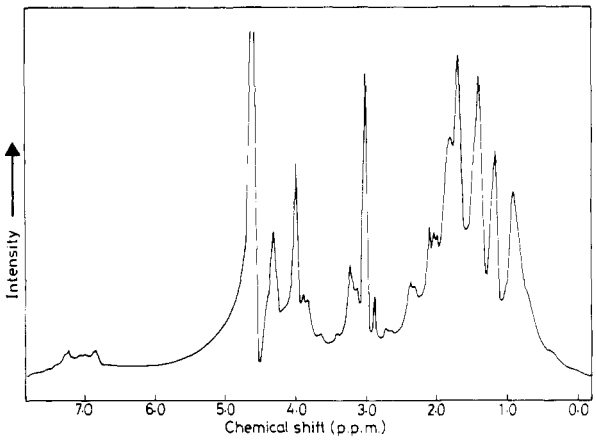

FIGURE 1: Proton NMR spectrum at 300 MHz of core histones in 2 M NaCl.

of an internal standard cancels out errors due to dilution, changes in viscosity, and instrument variation. Areas were reproducibly measured on different samples to ±7%.

The DNA concentration of core chromatin was determined by diluting an aliquot in 0.1% sodium dodecyl sulfate (SDS) and measuring the optical density at 260 nm using an extinction coefficient of 6600 per mol of nucleotide (Lee et al., 1963). Histone concentrations of core chromatin were determined by using the Folin reaction (Folin et al., 1927). Samples were measured against a standard of core histone whose concentration was determined as described above. The histone to DNA ratio determined in this way was 0.83. This may be compared to a value of 0.85 which can be calculated by assuming a molecular weight of 108 000 for a core histone octamer and by assuming that one octamer is associated with 212 base pairs of DNA in chicken chromatin. (Morris, 1976). The close similarity of these values together with the gel electrophoresis profile of the core chromatin histones, which shows the complete absence of H1, H5, and non-histone proteins and the presence of core histones in amounts similar to chromatin (Diaz & Walker, 1983), means that all proteins other than the core histones have been removed from the DNA.

The histone concentrations in the core chromatin used for NMR experiments were usually in the range 0.5–1.0 mg/mL. Higher concentrations of chromatin became too viscous to handle.

Sedimentation coefficients were measured in a Beckman analytical centrifuge equipped with ultraviolet optics and a photoelectric scanner. Circular dichroic spectra were measured in a Rousell-Jouan Dichrograph Mark II by using the procedures and precautions described previously (Henson & Walker, 1970).

Trypsin digestion of core chromatin was carried out as described previously (Diaz & Walker, 1983). The melting profile of core chromatin in 0.1 mM sodium phosphate and D_2O was obtained as described previously (Henson & Walker, 1970).

Results

The proton NMR spectrum of core histone in 2 M NaCl (Figure 1) is similar to those already described (Lilley et al., 1977; Cary et al., 1978). The resonances arise primarily from the side chains in the mobile tails. The area under the lysine resonance at 3.0 ppm was 80% of that expected from the concentration determined from the absorption spectrum. This difference may possibly be attributed to aggregation of the core histones in 2 M NaCl. The aggregates were observable

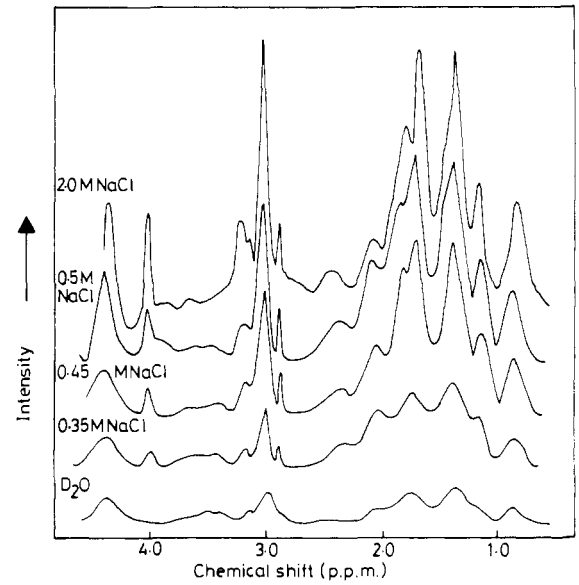

FIGURE 2: Proton NMR spectrum at 300 MHz of core chromatin in (from bottom to top) D_2O and 0.7 mM sodium phosphate, pD 7.0, and in D_2O and 0.7 mM sodium phosphate plus 0.35, 0.45, 0.5, and 2.0 M NaCl, respectively. Core histone concentration, 1.0 mg/mL; temperature, 297 K.

in the sedimentation boundary as a leading edge comprising 15–20% of the total protein. It appears that this aggregation affects the lysine side chains in such a way as to broaden the signal, causing loss of area. On the other hand, in 6 M urea, the area under the lysine resonance at 3.0 ppm was 98% of that expected from the concentration measured by optical absorption. This shows that the method of measuring concentrations of specific resonances in the NMR spectrum using an internal reference is quantitatively accurate when the resonance is relatively narrow.

By contrast, in 2 M NaCl, the arginine resonance at 3.25 ppm arising from the side chain $-CH_2$ is broader than the lysine ϵ-CH_2 resonance and considerably smaller in area despite there being 102 arginines as compared to 116 lysines per core histone octamer. The arginine side chains are less mobile than the lysines in the native octamer. This has been noted before (Nicola et al., 1978) and attributed to interaction between the C-terminal regions of H3 and H4 and the structured domains of H2A and H2B (Cary et al., 1978).

When the core chromatin was made 2 M in NaCl, the histones were completely dissociated from the DNA. The area of the lysine ϵ-CH_2 resonance at 3.0 ppm was measured relative to the internal standard and the total concentration of core histone determined by assuming that there are 116 lysines per octamer. The concentration estimated in this way was within 3% of the value determined independently from the Folin method described above. This shows that the lysine resonance at 3.0 ppm, measured immediately after dissociation in 2 M NaCl, represents all the lysines in the core chromatin. Thus, before aggregation has had a chance to occur, all lysine side chains can be accounted for in the spectrum and are relatively mobile. They are not, therefore, extensively involved in inter- or intramolecular salt linkages.

The proton NMR spectrum of core chromatin as a function of increasing NaCl concentration is shown in Figure 2. In D_2O and in 10 mM NaCl, the spectrum was almost featureless with a few broad peaks visible at 3.0, 1.7, 1.4, and 0.9 ppm. Between 0.1 and 0.2 M NaCl, the core chromatin became gelatinous and eventually precipitated from solution. The spectrum broadened still further, and the maxima seen at low salt almost disappeared. As the salt concentration was raised

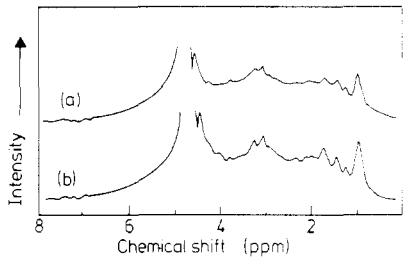

FIGURE 3: Proton NMR spectrum at 300 MHz of core chromatin in D_2O and 0.7 mM sodium phosphate, pD 7.0: (a) after treatment with trypsin followed by dialysis; (b) as in (a) but then made 0.6 M in NaCl.

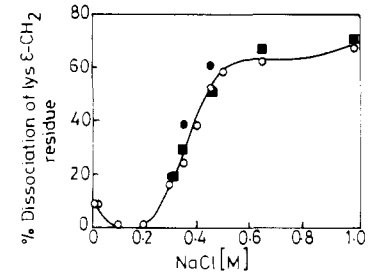

FIGURE 4: Variation of the lysine ϵ-CH_2 spectral peak at 3.0 ppm as a function of [NaCl]: (○) area of peak; (■) height of peak maximum; (●) area of peak on reverse curve after core chromatin had been made 2 M in NaCl and then dialyzed to the appropriate NaCl concentration.

above 0.2 M, the chromatin redissolved, and relatively narrow spectral lines appeared with major bands centered at 4.0, 3.2, 3.0, 1.7, 1.4, and 0.9 ppm which increased in intensity with increasing salt concentration. Apart from broadening caused by the high viscosity of the solution, the spectrum in 0.6 M NaCl was very similar to the spectrum of core histone (Figure 1) and suggests that the spectral lines seen in high salt arise from the amino- and carboxyl-terminal "tails". This was confirmed by trypsin digestion as follows.

The effect of digesting core chromatin with trypsin for 18 h at room temperature under the conditions described previously is to degrade the basic amino- and carboxyl-terminal ends of each core histone to leave five large limit peptides. The degraded terminal regions dissociate from the core chromatin and can be removed by dialysis (Diaz & Walker, 1983). The spectrum of core chromatin in D_2O after trypsin digestion and dialysis is shown in Figure 3. When the salt concentration was increased to 0.6 M, the spectrum remained essentially unaltered, and the area of the spectral peak at 3.00 ppm, identified as that of lysine ϵ-CH_2, remained constant relative to the internal standard of TSP. The large increase in intensity of the peaks between 3.2 and 0.9 ppm which takes place with the native core chromatin was not observed. The sharp peaks which become visible in native core chromatin between 0.3 and 0.6 M NaCl may therefore be identified with the amino acid residues in the trypsin-sensitive amino- and carboxyl-terminal tails of the four core histones defined as follows. The composition of the trypsin-resistant peptides which remain bound to DNA was determined previously from their size and amino acid analysis (Diaz & Walker, 1983). More recently, the amino-terminal ends of the trypsin-resistant peptides have each been sequenced, and this has enabled us to define the trypsin-resistant peptides more precisely (J. Gagnon and I. O. Walker, unpublished results). From the amino-terminal sequences and the amino acid composition of each peptide, the trypsin-resistant peptides are defined as follows: H2A, residues 10–118; H2B, residues 31–116 and residues 31–108 in equal proportions; H3, residues 57–122; H4, residues 20–102. The amino acid sequences of the trypsin-sensitive regions are obtained by difference from the complete sequence of each histone. From this trypsin digest, the amino acid carboxyl-terminal ends of H2A, H2B, and H3 but only the amino-terminal end of H4 can be sequenced. There are thus a total of 14 basic tail domains which are sensitive to trypsin in each octamer.

The spectrum in the aromatic region between 6.0 and 9.0 ppm remained featureless at low salt concentrations. Very small peaks could be seen in 0.6 M NaCl between 6.5 and 7.5 ppm, suggesting that very few, if any, of the phenylalanine or tyrosine side chains became mobile on salt dissociation. This part of the spectrum was not investigated further. The low intensity of signal in this region of the spectrum is consistent with the conclusion that only the basic tails have dissociated in 0.6 M NaCl since they contain, at most, two tyrosines (Diaz & Walker, 1983).

The area of the ϵ-CH_2 resonance of lysine at 3.0 ppm is shown as a function of NaCl concentration in Figure 4. The area increased sigmoidally over the range 0.2–0.6 M, where a plateau was reached. Between 0.6 and 2.0 M NaCl, the salt range over which the core histones completely dissociate from DNA (Burton et al., 1978), the area increased yet again. Taking the area in 2 M NaCl as 100% dissociation and as representing the total concentration of dissociated lysine side chains in the core histone and taking the absence of a signal at 0.2 M NaCl as zero dissociation, Figure 4 shows that 61 ± 5% of the lysines have dissociated at the plateau in 0.6 M NaCl.

Core chromatin was made 2 M in NaCl to completely dissociate the histones from the DNA. The salt concentration was then decreased by dialysis to values within the dissociation range and the area of the lysine peaks measured. The association curve could not be distinguished, within experimental error, from the dissociation curve (Figure 4). This shows that the lysine ϵ-CH_2 group dissociates from and reassociates with chromatin in a thermodynamically reversible reaction. The same conclusion can be drawn for the other major peaks in the spectrum since the complete spectra are superimposable after complete dissociation and reassociation.

The half-width of the peak at 3 ppm remained constant at 40 Hz over the salt range 0.3–0.6 M, and the peak height, measured at the maximum, showed the same sigmoid dependence on salt concentration as the peak area (Figure 4). These two observations, taken together, imply that the signal which develops with increasing NaCl concentration represents a single spectral species and that the dissociation of the lysine ϵ-CH_2 peak can therefore be represented by a two-state process: the bound state has an NMR spectrum which is very broad whereas the dissociated state has a narrow spectral line which indicates that the lysine residues are relatively mobile. The small, narrow peak at 2.8 ppm, tentatively identified as arising from N^{ϵ}-methyllysine, also increases in intensity over the salt range 0.2–0.6 M. Since this modified lysine residue is found in the amino-terminal tails of H3 and H4 (Ogawa et al., 1969; Brandt & Van Holt, 1974), these peptide sequences, at least, must be involved in the dissociation.

The other resonances between 3.2 and 0.9 ppm which increase in intensity with salt concentration show considerable overlapping and cannot be resolved. They must arise from the CH, CH_2, and CH_3 residues of the amino acids in the trypsin-sensitive tails which are rich in glycine, alanine, arginine, and lysine (Diaz & Walker, 1983). When the peak heights of the major resonances at 1.4, 1.7, and 0.9 ppm are displayed as a function of [NaCl] over the range 0.2–0.6 M,

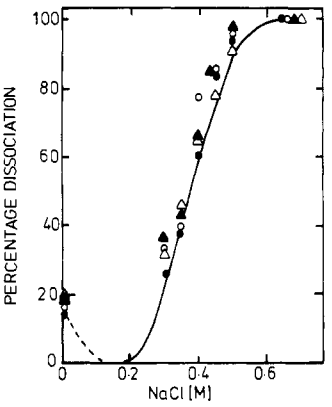

FIGURE 5: Variation of selected spectral peak heights as a function of [NaCl]: (---) Lys ϵ-CH_2 at 3.0 ppm; (●) N^{ϵ}-methyllysine at 2.8 ppm; (○) peak at 1.65 ppm; (△) peak at 1.4 ppm; (▲) peak at 0.9 ppm.

they also showed a sigmoid dependence on salt concentration and followed very closely the curve for the lysine ϵ-CH_2 resonance at 3 ppm (Figure 5). With the assumption that peak height is a true measure of peak area, as it is for lysine ϵ-CH_2 (cf. Figure 4), it may be concluded that all the resonances under the signals at 1.4, 1.7, and 0.9 ppm represent a single dissociated state and that the dissociation of the trypsin-sensitive tails is an all or none process. Either the tails are completely bound or they are dissociated; they do not dissociate by a zipper mechanism (Burgen et al., 1975). Assuming that the area under the lysine ϵ-CH_2 resonance at 3 ppm in 2.0 M NaCl represents the total number of lysines in the core histone complex, then 61% of the lysines are dissociated in 0.6 M NaCl. This figure agrees very well with the number of lysines (60%) contained in the trypsin-sensitive tails. These cleavage sites were identified on the basis of amino acid composition and peptide size. More recent data derived from sequencing the amino-terminal end show that peptide T3 [see Diaz & Walker (1983) for definition of these peptides] is derived from H2B by cleavage at Lys-30 rather than Lys-31 (I. O. Walker, unpublished results). Thus, the major conclusion is that the basic tails of all the core histones are completely dissociated between 0.2 and 0.6 M NaCl.

Since the dissociation can be represented by a two-state system, it is possible to write the reaction as follows:

$$A + n\text{NaCl} \rightleftharpoons D \qquad (1)$$

where A is the number of moles of associated peptide, D is the number of moles of dissociated peptide at a given NaCl concentration, and n is the number of moles of NaCl bound to the dissociated species. If K is the equilibrium constant for the reaction in eq 1

$$K = \frac{(D)}{(A)(\text{NaCl})^n}$$

and

$$\log K = \log \frac{(D)}{(A)} - n \log (\text{NaCl}) \qquad (2)$$

where the terms in parentheses represent the activities of the reacting species. Since the activity coefficients of D and A are not known and since they are present in low concentrations, it is assumed that activity equals concentration. The ratio $(D)/(A)$ can be measured from the dissociation curve shown in Figure 4, where $(D) = \alpha$, the degree of dissociation of the Lys ϵ-CH_2 residue, $(A) = 1 - \alpha$, and $\alpha = 100\%$ at 0.6 M NaCl.

From eq 2, the slope of the curve of log $[\alpha/(1 - \alpha)]$ against log [NaCl], where [NaCl] is the concentration in moles per liter, gives n and the intercept gives K. This is shown in Figure 6. The variation is linear over the entire salt range and gives $n = 7 \pm 1$ and $K = 400$. Since the standard change in free energy ($\Delta G°$) equals $-RT \ln K$, $\Delta G° = 3.6$ kcal/mol under standard conditions of 1 M NaCl. The small value of $\Delta G°$ suggest that only electrostatic links are involved. The apparent invariance of n over the entire dissociation range shows that the average number of sodium chloride molecules bound to the dissociated species does not depend on the degree of dissociation. This is consistent with the behavior of a two-state, cooperative system. Furthermore, it implies that each tail has an equal probability of dissociating at a given salt concentration. Since the individual tails differ in both size and amino acid composition, this means that these factors do not determine the probability of dissociation.

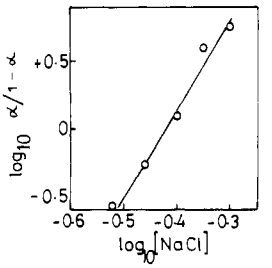

FIGURE 6: Variation of $\log_{10} [\alpha/(1 - \alpha)]$ with $\log_{10}$ [NaCl] for the Lys ϵ-CH_2 resonance at 3.0 ppm.

According to the theories of Record et al. (1978) and Manning (1978), n is related to the number of salt links (α) involved in the binding reaction to DNA by the equation $n = 0.88\alpha$. Applying this equation to the result obtained above and allowing that arginine behaves like lysine in the salt dependence of its binding to DNA give the number of salt links as 8 ± 1. The highly cooperative nature of the system implied by the demonstration of a two-state system and the sigmoid nature of the binding curve suggest that the number of salt linkages may be identified with the minumum size of the cooperative unit. The nature of the cooperative unit cannot be directly determined from the experiments reported here. At one extreme, the maximum size of the cooperative unit is the sum of the tails in the core histone octamer. On this view, all 14 tails of a given histone octamer (Diaz & Walker, 1983) dissociate together in a concerted reaction. Since there is a total of 103 basic side chains in the octamer tails and since there are only 8 salt links involved in the binding, the conclusion must be that only approximately 10% of the basic side chains are involved in direct interaction with DNA (less than 1 per basic tail) or the basic side chains make fractional interactions with each phosphate. It is difficult to reconcile either proposal with the observation that all the lysine and arginine side chains are highly immobile. Furthermore, there is evidence which shows that the lysine-rich histone tails can dissociate independently of the arginine-rich histone tails (Cary et al., 1978).

At the other extreme, the cooperative unit may be identified as an individual tail. On this view, each tail dissociates independently of the others in the octamer. This seems a more likely situation than the one discussed previously because cooperativity between tails implies that there are interactions between them requiring a minimal amount of structure whereas the evidence suggests that the tails are structureless (Diaz & Walker, 1983).

The average number of positively charged side chains in a tail is 7.3 (ignoring partly acetylated lysines). This agrees quite well with the number of salt links deduced experimentally and suggests that all the basic groups in the tails completely

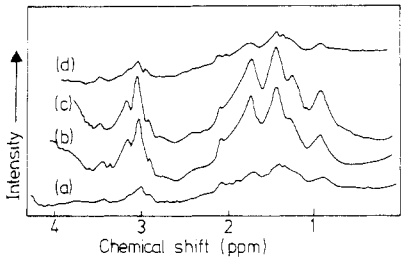

FIGURE 7: Proton NMR spectrum of core chromatin in D_2O and 0.1 mM sodium phosphate, pD 7.0, as a function of temperature: (a) 24 and 45 °C; (b) 55 °C; (c) 65 °C; (d) heated to 65 °C and then cooled to 24 °C.

neutralize an equivalent number of phosphate groups on the DNA. Thus, the 103 basic side chains in the tails interact with 103 phosphates or 51 base pairs out of the 212 base pairs associated, on average, with each core histone–DNA complex in chicken core chromatin.

Effect of Temperature. Increasing the temperature of core chromatin from 20 to 45 °C in 0.4 M NaCl where the tails are approximately half-dissociated did not alter the shape or intensity of the spectrum, and the area of the lysine $-CH_2$ peak remained constant. This suggests that the enthalpy of dissociation, ΔH, over this limited temperature range is zero. Thus, the dissociation reaction is entropically driven, which is consistent with the suggestion that only electrostatic salt links are involved. Since $\Delta G = \Delta H - T\Delta S$, the entropy of dissociation is −12 eu in 1 M NaCl at 297 K.

The melting profile of core chromatin in D_2O and 0.1 mM sodium phosphate was apparently biphasic with T_m values of 70 and 82 °C. The curve was not analyzed in detail, and it is important only to note that no hyperchromism was detected below 65 °C. When a sample of core chromatin in D_2O and 0.1 mM sodium phosphate was heated to 45 °C, no change occurred in the NMR spectrum. When heated to 55 °C, however, the sharp spectrum characteristic of dissociated tails was observed, and on further heating to 65 °C the spectrum remained unchanged (Figure 7). On cooling, the spectrum reverted to that observed at room temperature. Between 45 and 55 °C, the premelting transition in the thermal denaturation profile of core chromatin takes place (Wilhelm et al., 1974). In core particles, this premelting transition is reversible and is associated with a change in conformation of the DNA possibly resulting from the loss of supercoiling (Weischet et al., 1978). The area of the signal which develops between 45 and 65 °C is 65% of the area present in 2 M NaCl at room temperature. Clearly, the same proportion of lysines which dissociate in 0.6 M NaCl at room temperature has also dissociated in low salt at 55 °C. Thus, all the tail regions are completely dissociated in the thermal dissociation reaction which takes place over the same temperature range as the premelting transition in the DNA.

CD and Sedimentation. The CD spectrum of core chromatin in 0.6 M NaCl was identical with the spectrum in 0.7 mM sodium phosphate over the range from 320 to 200 nm. This shows that there has been no change in the secondary structure of the histones as the tails dissociate, which is consistent with our previous conclusion that the tails have no secondary structure (Diaz & Walker, 1983). The CD near-UV band of DNA, centered at about 280 nm, is sensitive to salt concentration and decreases in intensity with increasing salt, especially over the range studied here (Henson & Walker, 1970). However, no decrease in the intensity of this band was seen in core chromatin. This could be interpreted to mean that the decrease expected for free DNA is balanced by an increase in the intensity of the core chromatin associated with the release of the bound tails. On this view, the dissociation of the tails leads to a decrease in supercoiling.

Table I: Sedimentation Coefficient of Core Chromatin as a Function of NaCl Concentration[a]

[NaCl] (M)	s_{app} (S)	[NaCl] (M)	s_{app} (S)
0	25	0.45	77
0.01	48	0.50	66
0.35	84		

[a] All measurements were made at 20 °C in 0.7 mM sodium phosphate, pH 7.0, to which NaCl was added to the required concentration.

The sedimentation coefficient of the core chromatin complex as a function of salt concentration is shown in Table I. All values were determined at the same concentration of chromatin (40 μg/mL DNA) but were not corrected for solvent viscosity. The sedimentation coefficient increased between 0 and 10 mM NaCl from 25 to 48 S. Above this salt concentration, the chromatin tended to precipitate and did not dissolve to give a true solution until 0.20–0.30 M NaCl. In 0.35 M NaCl, the sedimentation coefficient had increased still further to 84 S. Between 0.35 and 0.5 M NaCl, it then decreased. With the assumption that the changes in the sedimentation coefficient are reflecting a change in the frictional coefficient, these observations show that the core chromatin molecules become more compact as the salt concentration increases up to 0.35 M. This folding is almost certainly due to a change in conformation of the DNA. By the same argument, over the salt range where the basic tails dissociate, from 0.35 to 0.5 M NaCl, the chromatin becomes less compact as a result of unfolding of the chromatin DNA. This unfolding is consistent with a decrease in the amount of supercoiling of the DNA.

Discussion

The method used here to prepare core chromatin involves relatively high salt concentrations to dissociate H1 and H5. When chromatin is subjected to these concentrations of salt, the core histones become mobile and slide along the DNA, and they then occupy positions on the DNA which do not necessarily correspond to their positions in the native structure (Burton et al., 1978). It could be argued, therefore, that the dissociation process describe here is not relevant to the native structure. This question can only be settled by carrying out similar studies on core chromatin prepared in such a way that the core histones retain their original positions on the DNA. Such a study is in progress, and the results will be presented elsewhere. However, since it is shown here that the bound and dissociated tails are in reversible thermodynamic equilibrium with DNA in core chromatin where sliding has taken place, then it must be concluded that in core chromatin where sliding has not occurred, either the tails are in the same equilibrium state or the tails are in a metastable state. If the latter is true, a thermodynamic analysis of the process will not be possible. The significance of the results presented here lies in their relation to a previous study which used salt to dissociate the histones completely (Burton et al., 1978). A comparison of the two studies allows conclusions to be drawn about the number and disposition of the salt linkages in the nucleosome and core particle as shown below.

The histone basic tails, defined operationally here as the regions comprising those peptides which can be degraded by trypsin and then removed by dialysis from the chromatin complex, dissociate from DNA over a lower salt concentration range than that required to remove the histones completely. Dissociation is highly cooperative, and the cooperative unit

appears to be the complete tail. The individual tails are either completely bound or completely dissociated. There is no intermediate conformational state corresponding to a partly bound tail. The tails are, in the dissociated state, largely structureless and flexible. However, they are not random coils, and the arginine side chains in particular appear to be in a more restricted conformation than the lysine side chains. The DNA, the other half of the binding site, undergoes a conformational change on dissociation which involves a loss of supercoiling. It is possible, therefore, that the tails are necessary to maintain the supercoiled state of the DNA in those regions where the tails bind. This is consistent with the effect of trypsin on core chromatin, which results not only in degradation and dissociation of the tails but also in uncoiling of the DNA (Diaz & Walker, 1983). Given that the cooperative unit is a complete tail, the number of salt links determined from the analysis of the dissociation is eight, and this implies that each basic side chain in the tails, both arginine and lysine, makes a salt link to a phosphate on the DNA. Thus, the histone tails in each core histone octamer are involved in a total of 103 salt links to DNA phosphate.

These same salt links are also broken when the temperature is increased from 45 to 65 °C, the temperature range over which the premelting transition occurs in chromatin. Thus, dissociation of the basic tails takes place before denaturation and strand separation of the DNA and is concomitant with the premelting transition. The premelting transition associated with the thermal denaturation of core particles is thought to involve the unsupercoiling of about two turns of double-helical DNA from either end (Simpson & Shindo, 1979). A similar conformational change appears to take place during the premelting transition of core chromatin. Thus, the thermal dissociation of the tails is concomitant with the unfolding or unwinding (but not denaturation) of at least two turns of double-helical DNA at the ends of the core particle plus, in the case of core chromatin, an unknown number of turns in linker DNA.

Since the tails have dissociated completely at salt concentrations below those required to completely dissociate the core histones, the structured domains of the core histone complex must interact strongly with DNA. As shown previously, these interactions involve, either directly or indirectly, α-helical segments in the core histones (Diaz & Walker, 1983). The thermodynamic analysis of the dissociation which was previously described by Burton et al. (1978) must therefore apply to the interaction of the structured part of the core histones with DNA and not the tails, as previously assumed. The salt links which are broken on the complete dissociation of H2A–H2B dimers and H3–H4 tetramers between 0.7 and 2 M NaCl therefore involve only the basic side chains in the structured, trypsin-resistant sections of these histone polypeptide chains. From the data in Burton et al. (1978), it can be shown that the number of salt links involved in binding one H2A–H2B dimer to core chromatin is 34 and that there are 32 per H3–H4 tetramer. Thus, there is a total of 100 salt links per octamer. The structured domains of the octamer have a total of 106 lysine and arginine side chains. Therefore, within the limits of the analysis, all the basic side chains in the structured domains make salt links to DNA. If the ~100 DNA phosphates involved in this interaction are situated on the inner face of the DNA which is supercoiled around the core histone octamer, approximately 100 base pairs may bind to the structured domains. It is interesting to note that a detailed analysis of the thermal melting of core particles has shown that about 100 base pairs in the middle of the core particle are stabilized by binding to the structured regions of the core histones (Weischet et al., 1978; McGhee & Felsenfeld, 1980).

At the ionic strength thought to prevail in the nucleus (0.1–0.2 M), all the tails are bound. There is thus a total of 203 salt links made to DNA per core histone octamer, that is, 100 made by the structured domain and 103 made by the 14 basic tails. If each octamer is associated, on average, with 212 base pairs in chicken chromatin (the chromatin repeat length), then every base pair in the repeat makes, on average, one salt link to core histone proteins. Furthermore, virtually all the basic side chains in the core histone complex are bound to DNA through salt links.

The conclusions reported here contrast markedly with those of another study of salt links in core particles based on an analysis of melting curve data (McGhee & Felsenfeld, 1980). In this study, the results suggested that only 15% of the phosphates in the DNA terminal regions were involved in interactions with histones, and it has been postulated that this low degree of neutralization may be representative of all core particle DNA. However, these conclusions were based on the assumption that melting of the DNA ends and release of bound histone took place simultaneously. This assumption would appear to be unjustified in light of the observations reported here which show that dissociation of the tails takes place at temperatures below that required for melting and during the premelting transition. The melting curve analysis is therefore concerned with the core DNA ends from which the histone tails have already dissociated. This being so, it would explain why the core DNA ends appear to have few interactions with histone and why trypsin treatment has no effect on the analysis.

The apparent free energy of binding one basic tail to DNA (ΔG) in 0.2 M NaCl (assumed to be the ionic strength of the nucleus) may be calculated from the van't Hoff isotherm as before (Kumar & Walker, 1980) and gives ΔG = 10.3 kcal/mol. For each octamer, there are 14 tails; therefore, the total free energy of tail binding is 144 kcal/mol. The original estimate for the binding free energy of a histone octamer to DNA in 0.2 M NaCl may now be revised to include the contribution from the tails. For the structured domain, $\Delta G'$ = 78 kcal/mol (Kumar & Walker, 1980). Therefore, for each octamer, $\Delta G'$(total) = $\Delta G'$(structural domain) + ΔG(tails) = 144 + 78 = 222 kcal/mol. This is the free energy required to dissociate an octamer of core histones under physiological salt conditions. This requirement may be reduced if the binding of the tails were weakened by chemical modification such as acetylation of the lysines, which has been associated with the activation of chromatin for transcription (Allfrey et al., 1964) or replication (Sung & Dixon, 1970). It is clear, however, that even complete dissociation of the tails does not lead to dissociation of the histones. Nevertheless, dissociation of the tails causes the DNA supercoil to unfold, and this conformational change may play a part in activating the nucleosome for biological function. For example, if the tails bind to base pairs at either end of the nucleosome (Cary et al., 1978), dissociation of the tails will result in unfolding of the DNA at these ends. This, in turn, will destroy the binding site for histone H1, which appears to require two full turns of DNA, or three supercoiled strands, to bind properly. If H1 cannot bind, the chromatin will not be able to fold into higher order conformations but will necessarily remain in an extended conformation. This may be a prerequisite for biological activity.

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References
Allfrey, V. G., Faulkener, R. M., & Mirsky, A. E. (1964) *Proc. Natl. Acad. Sci. U.S.A. 51*, 786-794.
Beaudette, N. V., Fulmer, A. W., Okabayashi, H., & Fasman, G. D. (1981) *Biochemistry 20*, 6526-6535.
Bradbury, E. M., Carpenter, B. G., & Rattle, H. W. E. (1973) *Nature (London) 241*, 123-127.
Brandt, W. F., & von Holt, C. (1974) *Eur. J. Biochem. 46*, 419-429.
Burton, D. R., Butler, M. J., Hyde, J. E., Phillips, D., Skidmore, C. J., & Walker, I. O. (1978) *Nucleic Acids Res. 5*, 3643-3664.
Cary, P. D., Moss, T., & Bradbury, E. M. (1978) *Eur. J. Biochem. 89*, 475-482.
Davies, K. E., & Walker, I. O. (1974) *Nucleic Acids Res. 1*, 129-139.
Diaz, B. M., & Walker, I. O. (1983) *Biosci. Rep. 3*, 283-292.
Folin, O., & Ciocalteu, V. (1927) *J. Biol. Chem. 73*, 627.
Henson, P., & Walker, I. O. (1970) *Eur. J. Biochem. 16*, 524-534.
Kumar, N. M., & Walker, I. O. (1980) *Nucleic Acids Res. 8*, 3535-3553.
Lee, M. F., Peacocke, A. R., & Walker, I. O. (1963) *Biochim. Biophys. Acta 72*, 310.
Lilley, D. M. J., Pardon, J. F., & Richards, B. M. (1977) *Biochemistry 16*, 2853-2860.
Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179-246.
McGhee, J. B., & Felsenfeld, G. (1980) *Nucleic Acids Res. 8*, 2751-2769.
Morris, N. R. (1976) *Cell (Cambridge, Mass.) 9*, 627-632.
Moss, T., Cary, P. D., Aberchrombie, B. D., Crane-Robinson, C., & Bradbury, E. M. (1976a) *Eur. J. Biochem. 71*, 337-350.
Moss, T., Cary, P. D., Crane-Robinson, C., & Bradbury, E. M. (1976b) *Biochemistry 15*, 2261-2267.
Nicola, N. A., Fulmer, A. W., Schwartz, A. M., & Fasman, G. D. (1978) *Biochemistry 17*, 1779-1785.
Ogawa, Y., Quagliarotti, G., Jordan, J., Taylor, C. W., Starbuck, W. C., & Busch, H. (1969) *J. Biol. Chem. 244*, 4387-4392.
Record, M. T., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103-178.
Ruiz-Carillo, A., & Jorcano, J. L. (1979) *Biochemistry 18*, 760-768.
Simpson, R. T., & Shindo, H. (1979) *Nucleic Acids Res. 7*, 481-492.
Sung, M. T., & Dixon, G. H. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 1616-1623.
Weischet, W. O., Tatchell, K., Van Holde, K. E., & Klump, H. (1978) *Nucleic Acids Res. 5*, 139-160.
Whitlock, J. P., & Stein, A. (1978) *J. Biol. Chem. 253*, 3857-3861.
Wilhelm, F. X., De Murcia, G. M., Champagne, H. M., & Duane, M. P. (1974) *Eur. J. Biochem. 45*, 431-443.

Major Oligosaccharides in the Glycoprotein of Friend Murine Leukemia Virus: Structure Elucidation by One- and Two-Dimensional Proton Nuclear Magnetic Resonance and Methylation Analysis†

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ABSTRACT: The highly microheterogeneous, *N*-glycosidically linked oligosaccharides in the glycoproteins of Friend murine leukemia virus (as produced by Eveline cells) were liberated with endo-β-*N*-acetylglucosaminidase H and by alkaline hydrolysis. They were fractionated (as desialylated oligosaccharitols) by gel filtration and by concanavalin A affinity chromatography, and the major fractions were analyzed by methylation–gas chromatography–mass spectrometry, by digestion with exoglycosidases, and, especially, by one- and two-dimensional proton nuclear magnetic resonance spectroscopy. Guidelines for qualitative and quantitative analysis of complex oligosaccharide mixtures by NMR were worked out and the results compared with those obtained by methylation analysis. It was found that these major fractions consist of bi-, tri-, and tetraantennary oligosaccharitols of the "complex" type (comprising a minority of species with *N*-acetyllactosamine repeating units), which are, in part, substituted by nonreducing terminal Galα(1→3) and/or bisecting GlcNAcβ(1→4) residues.

The surface of murine leukemia virus (MuLV)[1] particles is—like that of other *Retroviridae* and of enveloped viruses in general—studded with glycoprotein "spikes" protruding to the exterior (Weiss et al., 1982). The amino acid sequences of several MuLV glycoproteins, as occurring on different viral strains, have been established [e.g., Chen (1982), Koch et al.

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[1] Abbreviations: A1, glycoprotein *N*-glycan of the N-acetyllactosaminic ("complex") type; BSA, bovine serum albumin; Con A, concanavalin A; endo H, endo-β-*N*-acetylglucosaminidase H; Gal, galactose; GalOH, galactitol; GLC, gas–liquid chromatography; Glc, glucose; GlcNAc, 2-acetamido-2-deoxyglucose; gp, glycoprotein; M, glycoprotein *N*-glycan of the mixed ("hybrid") type; Man, mannose; MS, mass spectrometry; MuLV, murine leukemia virus; F-MuLV, Friend strain of MuLV; NMR, nuclear magnetic resonance; 1D and 2D, one and two dimensional; NOE, nuclear Overhauser enhancement or effect; ppm, parts per million; SDDS, spin-decoupling difference spectroscopy.

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