Effect of DNA length on the nucleosome low salt transition

Ann E.Dieterich*, Hilbert Eshaghpour[†], Donald M.Crothers[†] and Charles R.Cantor*

*Barth Laboratory, Department of Chemistry, Columbia University, New York, NY 10027, and

[†]Department of Chemistry, Yale University, New Haven, CT 06520, USA

Received 6 February 1980

ABSTRACT

The effect of DNA length on the low salt unfolding transition of nucleosomes has been studied by the use of fluorescently labeled histones. Nucleosomes were formed by the reconstitution of bulk DNA fragments averaging 173 and 250 base pairs in length. These nucleosomes exhibited a conformational change in a transition centered at about 7 mM ionic strength, very different from that observed for the standard 145 bp nucleosomes (1-3mM). In addition, the conformational change of the 173 and 250 bp nucleosomes involves twice as many ions as that of the 145 bp nucleosomes.

INTRODUCTION

Eukaryotic chromatin exists in a variety of structural states in vivo. Some of these states can be related to the functional state of underlying portions of the genome (1-3). Conformational changes in isolated chromatin and nucleosomes have been studied by a variety of physical and chemical methods (4,6). We have previously reported the use of covalently bound fluorescent dyes in studying the structural states of isolated nucleosomes (7-9). These studies were done with core nucleosomes, containing the fluorescent dye iodoacetyleneethylenediamine (1,5) naphthol sulfonate (IAEDANS), specifically bound to cysteine 110 of histone H3. One of the structural transitions we observed by fluorescence occurs when nucleosomes are placed in solutions of less than 5 mM ionic strength. This transition results in a dramatic structural opening in which the two molecules of H3 move apart, the nucleosome becomes flexible, and the cysteine of H3 becomes more exposed to the solution. Measurements of the translational (10) and rotational (11) frictional coefficients also show that nucleosomes become more extended in low ionic strength

solution. This is quite different from the compact, rigid form of the core nucleosome which exists in 10 to 100 mM ionic strength.

In current models of nucleosome structure, the DNA double helix is wrapped more than one full turn around the nucleosome core (12-14). Therefore, the low salt transition may result from electrostatic repulsion between the two turns of the DNA, where they lie close together on the surface of the nucleosome. If this is so, the position of the low salt transition should depend on the length of the nucleosomal DNA. Here we report further studies on the low salt transition of fluorescently labeled nucleosomes, in which DNA fragments of different lengths were used.

MATERIALS AND METHODS

Mononucleosomes were prepared from chicken erythrocytes and fractionated by precipitation with 0.1 M KCl (15) as previously described (7). The supernatant of the 0.1 M KCl precipitation was taken as 145 bp nucleosomes. Alternatively, 145 bp nucleosomes were prepared by micrococcal nuclease digestion of H1, H5 depleted chromatin (16). Nucleosomes containing 173 bp DNA were prepared from the 0.1 M KCl precipitate obtained as described above (15). The precipitate was redissolved in 10 mM Tris-HCl, 2 mM EDTA, pH 7.8 (TE). Histone H1 was removed by sedimenting these nucleosomes through 5-20% sucrose gradients containing 0.6 M NaCl and 5 mM EDTA, pH 7.8. This procedure yielded nucleosomes containing about 173 bp of DNA (17). The DNA size in our particles is slightly larger than the 160 bp reported for chromatosomes by Simpson (17), probably mainly because of a lesser extent of digestion. In some experiments, 145 and 173 bp nucleosomes isolated in this manner were labeled and reconstituted. In other experiments the DNA was isolated from 145 and 173 bp nucleosomes by phenol extraction followed by precipitation with ethanol. DNA of approximately 250 bp in length was obtained from spacerless dinucleosomes, prepared from H1, H5-depleted chromatin as previously described (16).

Histones were prepared from whole chicken erythrocyte chromatin by extraction with 0.2 N H_2SO_4 (18). Core histones free of H1 and H5 were obtained by precipitation with 0.5 M HClO_4 (19).

Labeling of nucleosomes with the fluorescent dye IAEDANS was carried out as previously described (7). IAEDANS was obtained from Molecular Probes, Inc. Tritium labeled IAEDANS was synthesized according to the procedure of Huang et al. and was provided by Dr. R.H. Fairclough (20). Histones were labeled in the same manner as nucleosomes, in 5 M urea, 2 M NaCl, 20 mM Tris, 4 mM EDTA, pH 8.0. High urea concentrations are required to prevent the histones from precipitating upon addition of the dye. Reassembly of 145 and 173 bp nucleosomes or reconstitution of 1:1 weight:weight mixtures of DNA and histones, was carried out as described previously (7). Nucleosome-size products were purified in 5-20% sucrose gradients containing 5 mM EDTA, pH 8.0. The 250 bp nucleosomes were formed by reconstitution of 250 bp DNA and a 1.5 x molar excess of core histones as described elsewhere (25). The monomeric 250 bp nucleosome, containing a single octamer of histones, was fractionated from spacerless dinucleosomes (16) by isokinetic sucrose gradient sedimentation (25). Histones were examined on 15% acrylamide gels containing sodium dodecyl sulfate, with a 5% stacking gel (21). DNA was examined on 5% acrylamide gels in Tris-Borate, EDTA buffer (22). The sizes of the DNA fragments were determined by comparison with a series of Hae III restriction fragments of ColE1 DNA (23).

Labeled nucleosomes were brought to low salt by dialysis against 0.1 M KCl, 1/50 x TE, followed by several changes of 1/50 x TE. The 0.1 M KCl was used to facilitate the dialysis of EDTA. During fluorescence experiments, the ionic strength was varied by addition of appropriate amounts of 10x or 100x stock solutions of TE at pH 7.8.

Fluorescence was measured using a Schoeffel RRS 1000 spectrofluorimeter, interfaced to a Tectronix E31 programmable calculator. Excitation was at 340 nm. The temperature in the sample compartment was maintained at 25°C with a circulating water bath.

RESULTS

Nucleosomes containing DNA fragments of various lengths and labeled with the fluorescent dye IAEDANS on cysteine 110 of histone H3 were prepared as described above. Three size classes

of DNA were used; with nominally 145, 173 and 250 base pairs (bp). The lengths of the DNA fragments were determined by polyacrylamide gel electrophoresis using a Hae III digest of ColE1 DNA as a standard (Figure 1). The 145 bp DNA sample contained fragments from 142 to 155 bp in length. The 173 bp DNA sample primarily contained fragments from 165 to 192 bp. The 250 bp

FIGURE 1

Five percent polyacrylamide DNA sizing gel. The DNA fragments from the three size classes of nucleosomes are shown (left to right): 250 bp, 173 bp and 145 bp. On the extreme right, a total Hae III digest of Col E1 DNA is shown as a standard. The visible bands are, from top to bottom,: 1100, 1050, 2x910, 2x440, 2x400, 250 and 178 base pairs in length. Electrophoresis is from top to bottom.

DNA sample contained molecules between 235 and 270 bp in length, with some higher molecular weight material at about 360 bp.

We have previously shown the nucleosomes, reconstituted from 145 bp DNA and labeled histone, and purified by the methods used here are indistinguishable from native nucleosomes by the criteria of sucrose gradient sedimentation, pancreatic DNase I digestion pattern (7), electrophoretic mobility, and molar ellipticity at 282 nm (9). The samples used in these experiments were also native by the criteria of DNase I digestion pattern and sedimentation properties.

When varying amounts of a Tris-EDTA stock solution at pH 7.8 are added to 145 bp nucleosomes in low salt (1/50 x TE), the titration curve shown in Figure 2 is obtained. The curve extends from about 0.5 to 10 mM ionic strength, with a midpoint at about 3 mM. For 173 and 250 bp nucleosomes, the titration curves extend from 2.0 to 20 mM ionic strength with midpoints at 7 mM. Thus, longer DNA appears to stabilize the low salt form of the nucleosome.

As the basic physical difference between 173 and 250 bp

FIGURE 2

Titration of IAEDANS-labeled nucleosomes at low ionic strength, showing the effect of DNA length. IAEDANS fluorescence is given as percent of the initial fluorescence at very low ionic strength (1/50 x TE). Excitation is at 340 nm, emission at 476 nm. The titration curves of the three size classes of nucleosomes are shown: —●— 145 bp, --○-- 173 bp, and ...▲... 250 bp nucleosomes.

nucleosomes and the 145 bp sample is the amount of negatively charged DNA bound by the histones, it is likely that the return to the compact form requires the binding of additional positive ions.

The fluorescence emission spectra of the nucleosomes containing 173 and 250 bp of DNA are similar to those of the 145 bp sample. The λ_{max} of fluorescence in low salt is about 490 nm for all three samples, and in TE it is 476 nm. This supports the notion that the structures of the two forms are insensitive to DNA lengths. The differences in response of the 145 and 173 bp nucleosomes to addition of buffers do not result from differences in histone composition or modification since the same behavior is seen whether nucleosomes are reconstituted from isolated DNA and bulk histones, or from dissociated samples of nucleosomes.

Since the midpoint of the transition is the same for 173 and 250 bp nucleosomes, the critical DNA length affecting the position of the transition must be between the lengths of the 145 and 173 bp samples. From the titration curves (Figure 2) it is clear that 173 bp nucleosomes contain no material titrating like the 145 bp sample. The titration curve of 145 bp nucleosomes is centered at 3 mM ionic strength, at which ionic strength the transition of the 173 bp sample has barely begun. Similarly, the titration curve of 145 bp nucleosomes does not show more than one component, as it would if the sample contained an appreciable fraction of material titrating like 173 bp nucleosomes (Figures 2 and 3). Thus the critical DNA length is probably between 155 and 165 bp, of which neither sample contains an appreciable amount.

The magnitude of the fluorescence change which occurs in the low salt transition is smaller for 173 bp than for 145 bp nucleosomes and smaller still for 250 bp nucleosomes. This could arise from differences in the quantum yields of the samples, or from loss of material due to precipitation in the 173 or 250 bp samples at moderate ionic strengths. Quantum yields in moderate and low ionic strengths of 145 and 250 bp nucleosomes were compared, using samples prepared from histones labeled with ^3H -IAEDANS. The results are displayed in Table 1.

FIGURE 3

Normalized fluorescence titration curves of 145, 173 and 250 bp nucleosomes. Fluorescence at 476 nm is given as ΔF_{rel} , defined in the text. Two sets of data are shown for each size class of nucleosomes. They are: circles, 145 bp nucleosomes (—); triangles, 173 bp nucleosomes (----); and squares, 250 bp nucleosomes (— — —).

The quantum yields of the 145 and 250 bp samples are the same. However, the concentration of the 250 bp sample decreases in 30 mM ionic strength, as shown by the decrease in $^3\text{H-cpm}/100\lambda$. That of the 145 bp sample does not. This entirely accounts for the difference in the fluorescence changes of the two samples (last column of Table 1). In a second experiment, a similar but smaller loss of material was observed in the 173 bp sample. The loss of material in the nucleosomes containing longer DNA may result either from low solubility of intact nucleosomes or incorrect refolding of nucleosomes at moderate ionic strength, resulting in precipitation.

We have observed considerable variability in the overall fluorescence change from one sample to another. Within a single experiment, however, the relative fluorescence changes of 250, 173 and 145 bp nucleosomes remain the same. Different experiments may be compared by normalizing the fluorescence changes at each ionic strength relative to the maximum range in fluores-

Table 1. Fluorescence intensity of nucleosome samples at different ionic strength.

Nucleosome	³ H-IAEDANS (cpm/100 μ l)		F ^a		F/cpm ^b		F ₂ /F ₁ ^c	(F/cpm) ₂ /(F/cpm) ₁
Sample ^c	1	2	1	2	1	2		
145 bp	76.2	77.6	162	254	2.13	3.27	1.57	1.54
250 bp	97.3	87.6	199	280	2.04	3.20	1.41	1.57
145-Std	97.5	99.8	295	292	3.02	2.93	.99	.97

a. Relative fluorescence F is determined by integrating the area under the emission spectrum of IAEDANS between 400 nm and 600 nm. Excitation was at 340 nm.

b. The fluorescence per cpm ³H is a measure of the relative quantum yield.

c. For the 145 bp and 250 bp, sample 1 is in 1/50 x TE; for the former, sample 2 is at 16 mM ionic strength; for the latter, sample 2 is at 30 mM ionic strength. For the 145-Std nucleosome, all measurements were made at 16 mM ionic strength. This sample serves to monitor the response of the fluorimeter from one series of experiments to the next.

cence. The quantity ΔF_{rel} is defined by:

$$\Delta F_{rel} = \frac{F_e - F_o}{F_m - F_o}$$

where F_o is the initial fluorescence in 1/50 x TE, F_m is the maximal fluorescence for that sample, in 1x to 2x TE, and F_e is the equilibrium fluorescence at a particular ionic strength. In Figure 3, data from two samples of each size class of DNA are plotted as ΔF_{rel} versus ionic strength. The two 250 bp samples are the one shown in Figure 2 and the one described in Table 1. Although the maximal fluorescence change of one was 25%, and of the other, 41%, the two sets of data agree very closely when fluorescence is plotted as ΔF_{rel} .

Using the same data presented in Figure 3, we calculated for each sample the apparent equilibrium constant K_o at intermediate ionic strengths. Since it is unlikely that histones are dissociating at such low ionic strengths, the overall reaction is probably a unimolecular conformational equilibrium of the type $N' \rightleftharpoons N$. Then the apparent K_o for the overall reaction (regardless of the presence of any intermediate steps) may be calculated by:

$$K_o = \frac{N'}{N} = \frac{F_e - F_m}{F_o - F_e}$$

Because the conformational equilibrium depends on the ionic

strength, the overall reaction is probably of the form $N' + n M^+ \rightleftharpoons N$. The average number of positive and negative ions involved in the reaction, n , may be obtained from (11,24):

$$\frac{\partial \log K_O}{\partial \log [M^+]} = -n$$

A plot of $\log K_O$ versus $\log [M^+]$ is shown in Figure 4. The slopes were, for 145 bp nucleosomes -2.2, for 173 bp nucleosomes -4.5, and for 250 bp nucleosomes -3.6. The last two slopes are the same within experimental error. If equal numbers of cations and anions are bound, $n/2$ is the number of salt molecules bound on refolding. At the other extreme, if only cation binding occurs, n cations are required to yield an average of $n/2$ for both ions. Thus the number of cations required to bring 145 bp nucleosomes to the compact form is 1-2, and for 173 and 250 bp nucleosomes it is 2-4.

In previous fluorescence studies of the low salt transition (8), we used NaCl to titrate core nucleosomes in 0.1 mM Tris-HCl, 0.02 mM EDTA, pH 7.0. The midpoint of the transition was reproducibly lower than reported here for 145 bp nucleosomes, falling at 1.0 to 1.2 mM ionic strength. The only differences in the experiments are in the titrant used, and the pH. Much of the ionic strength of the Tris-EDTA buffer comes from highly charged EDTA anions. However, as mentioned above, positive ions are probably the ones involved in the transition. Much of the apparent discrepancy between the two sets of data can be eliminated if only the molarities of the positive ions, Na^+ and Tris H^+ are considered. The results obtained in Figure 4, where only positive ion concentration was considered, agree very well with previous results obtained from NaCl titrations of 145 bp nucleosomes, where n was found to be 2.4 (8). However, the two curves are still not exactly alike. The differences probably arise from the difference in pH in the two sets of experiments.

Further complications in the unfolding process are indicated by comparison of the data in Figure 2 with earlier electric dichroism measurements of nucleosome unfolding (11). Those experiments, which employed a pH 8.0 EDTA-containing buffer similar (but not identical) to that used here, revealed a mid-

FIGURE 4

Salt dependence of the low salt transition. The apparent equilibrium constant K_0 for the conformational equilibrium $N \rightleftharpoons N'$ was calculated as described in the text. Here $\log K_0$ is plotted versus the logarithm of the calculated positive ion concentration. Data from the three size classes of nucleosomes are shown: —●— 145 bp, ---▲--- 173 bp, and -■- 250 bp nucleosomes.

point of about 1.3 mM ionic strength at 7°C for unfolding the 145 bp nucleosome. The disagreement with the data in Figure 2 seems outside the range of experimental error and may be due simply to the difference in temperature or a direct perturbation of the stability caused by the presence of the fluorescent probes. However, dependence of the observed transition midpoint on the method of detection is consistent with the conclusion of Martinson et al. (26) that unfolding is not a two-state process. Indeed, kinetic studies of the low salt transition reveal the presence of several significant intermediate states (27). Experiments to uncover more details about the unfolding process are

under way.

DISCUSSION

The results reported here demonstrate that the ionic strength at which the low salt conformational transition of nucleosomes occurs depends on the length of the nucleosomal DNA. The critical DNA length which determines the position of the titration curve is in the neighborhood of 155 and 165 bp. Above this range, the length of the nucleosomal DNA does not affect the position of the low salt transition any further.

The titration curves of the 173 and 250 bp nucleosomes are also much sharper than that of 145 bp nucleosomes, indicating an increase in the number of ions involved in the reaction. Analysis of the salt dependence of the reaction showed that 2-4 monovalent ions participate in the low salt transition of 173 and 250 bp nucleosomes, whereas only 1-2 participate in that of 145 bp nucleosomes. This is 0.5-1 additional ions per half nucleosome, or per asymmetric unit.

These results suggest a fundamental difference between nucleosomes containing less than about 155-165 bp of DNA and those containing more than 155-165 bp. Above 165 bp, nucleosome stability as monitored by fluorescence is relatively insensitive to increase in DNA length. These data are consistent with other indications that more than 145 bp of DNA can be specifically bound to a core particle, such as the report by Simpson (17) for 160 bp particles. In addition, we recently found (25) that a 178 bp restriction fragment is uniquely positioned in a reconstituted core particle, and that the entire DNA length is protected from nuclease digestion in the particle.

Our results should facilitate comparison among different preparations of nucleosomes. In their studies of the sedimentation properties of 145 bp nucleosomes, Gordon et al. (10) reported two transitions: one at 1-2 mM KCl and a second at 7-8 mM KCl. More recently, however, they have assigned the second transition to contaminating nucleosomes containing 160-200 bp of DNA (28, V.N. Schumaker, personal communication), in excellent agreement with our results.

The significance of the low salt transition is two-fold.

First, it shows how the nucleosome opens when a stress is applied to the DNA. Some of the biological processes in which chromatin is involved presumably involve stresses favoring extension of the DNA. Second, it is a transition in which opening but not complete dissociation of the nucleosome occurs. Studying this transition gives us information about the protein-protein (26) and protein-DNA contacts (9) which hold the nucleosome together. The greater stability of the low salt form of nucleosomes containing long DNA should be helpful in any further studies of the low salt form, because the ionic conditions required are less extreme.

ACKNOWLEDGEMENTS

This work was supported by grants GM 14825 (to C.R.C.) and GM 21966 (to D.M.C.) from the National Institutes of Health, and PCM 77-09254 (to C.R.C.) from the National Science Foundation.

REFERENCES

1. Weintraub, H. and Groudine, M. (1976) *Science* **193**, 848-856.
2. Garel, A. and Axel, R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3966-3970.
3. Franke, W.W., Scheer, U., Trendelenberg, M., Zentgraf, H. and Spring, H. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 755-772.
4. Felsenfeld, G. (1978) *Nature* **271**, 115-122.
5. Kornberg, R.D. (1977) *Annual Review of Biochemistry* **46**, 931-954.
6. Zama, M., Bryan, P.N., Harrington, R.E., Olins, A.L. and Olins, D.E. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 32-41.
7. Dieterich, A.E., Axel, R. and Cantor, C.R. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 199-206.
8. Dieterich, A.E., Axel, R. and Cantor, C.R. (1979) *J. Mol. Biol.* **129**, 587-602.
9. Eshaghpour, H., Dieterich, A.E., Cantor, C.R. and Crothers, D.M. (1980) *Biochemistry*, in press.
10. Gordon, V.C., Knobler, C.M., Olins, D.E. and Schumaker, V.N. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 660-663.
11. Wu, H.M., Dattagupta, N., Hogan, M. and Crothers, D.M. (1979) *Biochemistry* **18**, 3960-3965.
12. Finch, J.T., Lutter, L.C., Rhodes, D., Brown, R.S., Rushton, B., Levitt, M. and Klug, A. (1977) *Nature* **269**, 29-36.
13. Pardon, J.F., Worcester, D.L., Wooley, J.C., Cotler, R.I., Lilley, D.M.J., and Richards, B.M. (1977) *Nucleic Acids Research* **4**, 3199-3214.

-
14. Crothers, D.M., Dattagupta, N., Hogan, M., Klevan, L. and Lee, K.S. (1978) *Biochemistry* 17, 4525-4533.
 15. Olins, A.L., Carlson, R.D., Wright, E.B. and Olins, D.E. (1976) *Nucleic Acids Research* 3, 3271-3289.
 16. Klevan, L. and Crothers, D.M. (1977) *Nucleic Acids Research* 4, 4077-4089.
 17. Simpson, R.T. (1978) *Biochemistry* 17, 5524-5531.
 18. Panyim, S., Bilek, D. and Chalkley, R. (1971) *J. Mol. Biol.* 246, 4206-4215.
 19. Oliver, P., Sommer, K.R., Panyim, S., Spiker, S. and Chalkley, R. (1972) *Biochem. J.* 129, 349-353.
 20. Huang, K.H., Fairclough, R.H. and Cantor, C.R. (1975) *J. Mol. Biol.* 97, 443-470.
 21. Laemmli, U.K. (1970) *Nature* 227, 680-685.
 22. Maxam, A.M. and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* 74, 560-564.
 23. Eshaghpour, H. and Crothers, D.M. (1978) *Nucleic Acids Research* 5, 13-21.
 24. Record, M.T., Woodbury, C. and Lohman, T. (1976) *J. Mol. Biol.* 107, 145-158.
 25. Eshaghpour, H. and Crothers, D.M. (1980), submitted for publication.
 26. Martinson, H.G., Time, R.J. and Burch, J.B.E. (1979) *Biochemistry* 18, 1082-1094.
 27. Dieterich, A.E. and Cantor, C.R. (1980), submitted for publication.
 28. Gordon, V.C., Schumaker, V.N., Olins, D.E., Knobler, C.M. and Horowitz, J. (1979) *Nucleic Acids Research* 6, 3845-3858.