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INFLUENCE OF HISTONE H1 ON CHROMATIN STRUCTURE

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Summary

The influence of histone H1 with respect to the conformation of oligonucleosomes was investigated by studying transient electric birefringence (TEB). Whole chromatin exhibits a positive birefringence explained by an helical compact structure in which the superhelical axis of DNA is parallel to the long axis of the helical array. Birefringence of H1-lacking oligonucleosomes is negative and proportional to the number of nucleosomes in the chain.

So, the removal of H1 induces a transition to an extended "bead and bridge" structure in which 32 base-pairs of DNA connect two consecutive beads. The following scheme is then proposed for DNA : 195 bp (nucleosome repeat in rat liver) = 163 bp (associated with the histone core) + 32 bp (linker DNA).

Eukaryotic chromatin consists of a basic repeating subunit termed "the nucleosome" which may be described in terms of two major structural domains (1-5). One is the core particle containing 145 base-pairs of DNA wrapped around a compact histone octamer composed of two of each of the four smaller histones H2A, H2B, H3 and H4. The other is the linker DNA connecting adjacent nucleosomes, the length of which varies between and within different organisms (50 base-pairs in rat liver). Our knowledge of the location and structural role of the very lysine-rich histone H1 is not yet complete. H1 appears not to be a structural component of the nucleosome but to be located in the internucleosomal space and associated with a part at least of the linker DNA (6-9). In fact H1 is highly asymmetric : its central region assumes a globular structure while the ends form unstructured tails (10) which may present different binding properties to the DNA (11,12). On the other hand H1 is involved in the coiling or folding of nucleosome chains into higher orders of structure (12-19) perhaps by functioning as a cross-linker (13,14,20) or (and) by facilitating the stacking of nucleosomes (12,17,19). The packing of nucleosomes has been discussed in terms of a solenoidal structure, superbeads units or helical arrays whose diameters (200-300 Å) are about twice that of nucleosome (12,17,19,21-28).

We have previously reported transient electric birefringence (TEB) measurements on oligomeric chromatin subparticles. A sharp transition in the electro-optical properties was observed for the hexamer which appears to be a unit

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particle in chromatin superstructure (29). The present paper describes a study on H1-depleted chromatin with a view to examine the influence of H1 on the spatial arrangement of nucleosomes. Calculations are also made to estimate how many base-pairs of DNA are associated with histone H1.

Methods

Preparation of rat liver chromatin

Nuclei were isolated from rat liver (30) and suspended at a concentration of 3.10^8 nuclei/ml. Micrococcal nuclease (300 units/ml) digestion of chromatin in the presence of Ca^{2+} (1mM) was for 2 min. 30 sec. at 37°C. The reaction was terminated by adding EDTA (5mM). The nuclei were then pelleted and lysed in 0.2mM EDTA, 0.2mM PMSF (Phenylmethylsulfonylfluoride) pH 7.0 for 20 min. at 4°C. After centrifuging the suspension, the chromatin was recovered in the supernatant, 60 to 70 OD units of which being layered on an isokinetic sucrose gradient (5-28.2 % ; 1mM phosphate buffer, 1mM EDTA, pH 7.4) according to Noll (31). After a 20 hours centrifugation at 26 000 rpm in a SW 27 rotor, nucleosome oligomers up to 7 consecutive nucleosomes can be separated and analyzed using a gradient scanner (29).

Histone H1 was removed from nucleosome oligomers by the method of Bolund and Johns (32). H1 is adsorbed on the cation exchange resin AG 50W-X2 in the presence of 1mM phosphate buffer, 150mM NaCl, pH 7.4 : 0.2 ml of equilibrated resin was added per ml of chromatin solution. After gently stirring for four hours at 4°C, the suspensions were centrifuged and the supernatants were dialyzed against 1mM phosphate buffer, 0.2mM EDTA, pH 7.4.

Histones were extracted twice with 0.25 N HCl (final concentration) under a fast vortex mixing and recovered by sedimentation of precipitated DNA. The supernatant was dialyzed against bidistilled water, lyophilised and analyzed in 15 % polyacrylamide gel (33).

Electric birefringence measurements

The principle of the TEB method is to orientate the macromolecules by means of an electric field E (34). This orientation gives rise to a positive or negative birefringence Δn with $\Delta n = n_{//} - n_{\perp}$ where $n_{//}$ and $n_{\perp}$ are the refractive index of the solution in directions parallel and perpendicular to E. Δn is related to the optical phase retardation δ by :

$$\Delta n = \frac{\delta \lambda}{2\pi l} \qquad (1$$

where λ is the wavelength of the incident light in vacuum and l is the Kerr cell path length.

At low fields, Δn is proportional to E^2 (Kerr's law) and a specific Kerr constant B is defined as

$$B = (\Delta n_{eq}/\lambda c E^2)_{E \to o} \qquad (2$$

c is the solute concentration and Δn_{eq} is the steady state value of Δn.

The birefringence apparatus is described elsewhere (35) : it uses a stable and noise-free He-Ne laser with $\lambda = 6328.10^{-7}$ cm. Rectangular pulses having amplitudes up to 1200 volts were applied (with a duration varying from 40 to 300 µsec). The signals displayed on a fast oscilloscope were photographed.

Table I : Birefringence sign of rat liver chromatin

Positive Birefringence	- Histones (36) - Large nucleosome oligomers ($n \geq 7$) - Whole chromatin
Negative Birefringence	- DNA* - Core particle - Small nucleosome oligomers ($n < 6$) - H1 depleted chromatin

The hexanucleosome presents no birefringence.
*Recent studies on nucleosomal DNA (37) have never shown a reversal of the sign as reported for short fragments by Houssier *et al.* (40). This was perhaps due to partial induced field denaturation.

Results and discussion

The sign of electric birefringence(or dichroism)of chromatin subunits gives information about their orientation, this one being due to the orientation of DNA wrapped around the protein core. Table I summarizes the results obtained in our laboratory.

The negative sign of core particle and mononucleosome is now well established and implies superhelical axis of DNA parallel to the short axis of the nucleosome disk (or oblate ellipsoid) (29,38,39).

For whole chromatin and nucleosome oligomers, our results differ from those previously reported which always showed negative signal (38,40). In fact, the very close values of birefringence for whole and H1-depleted chromatin found by Houssier *et al.* (40) indicate for the first one the removal of a part at least of histone H1. Furthermore,that such a "whole chromatin" exhibits positive signals in presence of Mg^{2+} or Mn^{2+} support this notion. Divalent cations are indeed known to be implicated in the maintenance of higher orders of structure (17,24).

We observe positive signal of birefringence for whole chromatin containing its full complement of five histones with protein/DNA and A_{230}/A_{260} ratios of respectively 1.65 and 0.78. This result is in agreement with theoretical calculations assuming a superhelical arrangement of nucleosomes with DNA uniformly supercoiled (41). In this model, the superhelical axis of nucleosomes is parallel to the long axis of the helical array. The change of the sign observed for smaller oligomers ($n \leq 6$) could thus be explained by a modification of the oligomers organization for the shorter chains of nucleosomes (29).

The removal of histone H1 from whole chromatin either by the cation exchange resin treatment or by increasing the salt concentration (0.6 M NaCl) induces a reversal of birefringence which becomes negative. Two explanations can be proposed for this negative sign :

α) The compact structure is conserved with a modification of the orientation of the nucleosomes axis which could be thus perpendicular to that of the chromatin superstructure (41)

β) The compact structure is unfolded in an extended form with then a greater participation of the DNA to the total birefringence.

Electron microscopy datas are not consistent with the first hypothesis. Indeed, it has been observed that the compact structure with fibers of about 250 Å in diameter open up into a thin filament of 100 Å in diameter ; this one is described by the familiar "beads on a string" form in which the nucleosomes are connected by segments of DNA (12,18,24,42,43). This model is quite in agreement with the second hypothesis proposed above.

We verified this interpretation by studying TEB of H1-depleted oligomers which present a mean molar ellipticity $\{\theta\}^{20°C}_{282nm}$ of 3600 deg.cm^2.dmole^{-1}. Gel electrophoresis of the different fractions revealed no trace of histone H1.

For each oligomer, birefringence Δn is negative and proportional to optical density (in the range 0.6 to 4 A_{260}/ml) : so, it can be expressed in term of specific birefringence. No detectable change was noted in the optical signal when a number of pulses (about twenty) was applied and after a 5-6 days storage at 4°C.

Figure 1 shows the variation of specific birefringence as a function of subunits in the multimer (n) for two applied fields. The linearity observed con-

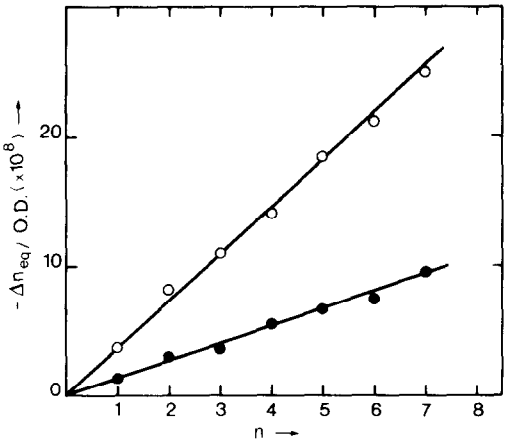

Figure 1 : Variation of steady state specific birefringence as a function of n, number of subunits in the fractions.
The electric fields applied were 3750 V/cm (○) and 2250 V/cm (●).

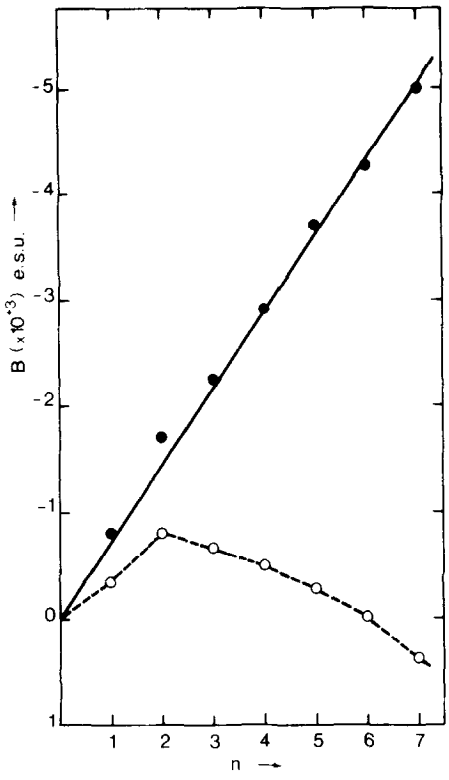

Figure 2 : Variation of the specific Kerr constant B as a function of n for H1-lacking (●) and H1-containing (○) oligonucleosomes.

firms the presence of an extended conformation for H1-depleted chromatin with the length of DNA participating to the birefringence proportional to the number of subunits.

In order to estimate the part of this DNA length which is no more protected by proteins, these TEB response curves are compared with those of H1-containing oligonucleosomes (29) by calculating the specific Kerr constants, B. For this purpose, the field dependence of Δn was studied for each oligomer. Linear relations were found between $\Delta n_{eq}/A_{260}$ and E^2 and B were determined from equation (2. Figure 2 shows the comparison of Kerr constants. For each oligomer, B increases when H1 is removed and the increase is proportional to n. This corresponds to the segment of DNA which was associated with H1 and now connects the beads, as revealed by electron microscopy of such fractions (Marion, Roux, De Murcia, unpublished results).

The increase of B has a mean value of -5.10^3 e.s.u. per nucleosome. Considering the Kerr constant per base-pair (bp) of DNA ($-0.18 \cdot 10^{-3}$ e.s.u.) (37), the bridge distance between two beads corresponds to 32 bp of DNA. This experimental result is in good agreement with enzymatic (42) and hydrodynamic (25) studies which lead to conclusion that H1 protects either 30 or 35 base-pairs of DNA

Our experiments show that the histone core of H2A, H2B, H3 and H4 can itself so protect 195-32 = 163 bp of DNA. Recent works on rat liver (43) or calf thymus (44) chromatin have respectively shown that 168 or 166 bp rather than 145, as described before, are originally associated with the core histones.

In conclusion,the removal of H1 from chromatin induces a transition from an helical compact conformation to an extended "bead and bridge" structure in which about 32 base-pairs of DNA connect two consecutive beads. So,we propose the following scheme for DNA in rat liver nucleosomes (195 bp) : 163 bp are associated with the histone core and 32 bp form the linker DNA, rather than respectively 145 and 50.

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References

1 - Kornberg, R.D. (1977) Ann. Rev. Biochem. 46, 931-954
2 - Felsenfeld, G. (1978) Nature, London, 271, 115-122
3 - Chambon, P. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 1209-1234
4 - Thomas, J.O. (1979) Companion to Biochemistry, Vol. 2, 78-108, Longman Inc., New York
5 - Rill, R.L. (1979) Molecular Genetics, Part III Chromosome structure, VI, 247-313, Academic Press,New York
6 - Whitlock, J.P. Jr. and Simpson, R.T. (1976) Biochemistry 15, 3307-3314
7 - Varshavsky, A.J., Bakayev, V.V. and Georgiev, G.P. (1976) Nucleic Acids Res. 3, 477-492
8 - Noll, M. and Kornberg, R.D. (1977) J. Mol. Biol. 109, 393-404
9 - Gaubatz, J.W. and Chalkley, R. (1977) Nucleic Acids Res. 4, 3281-3301
10 - Hartman, P.G., Chapman, G.E., Moss, I. and Bradbury, E.M. (1977) Eur. J. Biochem. 77, 45-51
11 - Singer, D.S. and Singer, M.F. (1976) Nucleic Acids Res. 3, 2531-2547
12 - Renz, M., Nehls, P. and Hozier, J. (1977) Proc. Nat. Acad. Sci. USA 74, 1879-1883
13 - Littau, V.C., Burdick, C.J., Allfrey, V.G. and Mirsky, A.E. (1965) Proc. Nat. Acad. Sci. USA 54, 1204-1212
14 - Bradbury, E.M., Carpenter, B.G. and Rattle, H.W.E. (1973) Nature 241, 123-125
15 - Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281-300
16 - Bellard, M., Oudet, P., Germond, J.E. and Chambon, P. (1976) Eur. J. Biochem. 70, 543-553
17 - Finch, J.T. and Klug, A. (1976) Proc. Nat. Acad. Sci. USA 73, 1897-1901
18 - Thoma, F. and Koller, Th. (1977) Cell 12, 101-107
19 - Worcel, A. and Benyajati, C. (1977) Cell 12, 83-100
20 - Lewis, E.A., De Buysere, M.S. and Rees, A.W. (1976) Biochemistry 15, 186-192
21 - Carlson, R.D. and Olins, D.E. (1976) Nucleic Acids Res. 3, 89-100
22 - Carpenter, B.G., Baldwin, J.P., Bradbury, E.M. and Ibel, K. (1976) Nucleic Acids Res. 3, 1739-1745
23 - Kiryanov, G.I., Manamshjan, T.A., Polyakov, V.Y., Fais, D. and Chentsov, Ju. S. (1976) FEBS Lett. 67, 323-327
24 - Vengerov, Y.Y. and Popenko, V.I. (1977) Nucleic Acids Res. 4, 3017-3027
25 - Schmitz, K.S. and Ramsay-Shaw, B. (1977) Biopolymers 16, 2619-2633
26 - Strätling, W.H., Müller, U. and Zentgraf, H. (1978) Exp. Cell Res. 117, 301-311
27 - Renz, M. (1979) Nucleic Acids Res. 6, 2761-2767

28 - Itkes, A.V., Glotov, B.O., Nikolaev, L.G., Preem, S.R. and Severin, E.S. (1979) Nucleic Acids Res. 8, 507-527
29 - Marion, C. and Roux, B. (1978) Nucleic Acids Res. 5, 4431-4449
30 - Hewish, D.R. and Burgoyne, L.A. (1973) Biochem. Biophys. Res. Comm. 52, 504-510
31 - Noll, M. (1974) Nature 251, 249-251
32 - Bolund, L.A. and Johns, E.W. (1973) Eur. J. Biochem. 35, 546-553
33 - Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346
34 - Fredericq, E. and Houssier, C. (1973) Electric Dichroism and Electric Birefringence, Clarendon Press, Oxford
35 - Bernengo, J.C., Roux, B. and Hanss, M. (1973) Rev. Sci. Inst. 44, 1083-1086
36 - Roux, B. (1976) Thesis, Lyon
37 - Marion, C. and Roux, B. submitted for publication
38 - Rill, R. and Van Holde, K.E. (1973) J. Biol. Chem. 248, 1080-1083
39 - Crothers, D.M., Dattagupta, N., Hogan, M., Klevan, L. and Lee, K.S. (1978) Biochemistry 17, 4525-4533
40 - Houssier, C., Bontemps, J., Emonds-Alt, X. and Fredericq, E. (1977) Ann. N.Y. Acad. Sci. 303, 170-189
41 - Rill, R.L. (1972) Biopolymers 11, 1929-1941
42 - Varshavsky, A.J., Bakayev, V.V., Chumackov, P.M. and Georgiev, G.P. (1976) Nucleic Acids Res. 3, 2101-2114
43 - Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell Biol. 83, 403-427
44 - Weischet, W.O., Allen, J.R., Riedel, G. and Van Holde, K.E. (1979) Nucleic Acids Res. 6, 1843-1862