Volume 3 no.8 August 1976 Nucleic Acids Research

Thermal denaturation of sheared and unsheared chromatin by absorption and circular dichroism measurements

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Received 19 April 1976

ABSTRACT

Thermal denaturation of chromatin is observed by simultaneously monitoring absorption and circular dichroism at 276 nm as functions of temperature. Either observation indicates that sheared chromatin shows less thermal stability than native chromatin. The temperature-dependent ellipticities at 276 nm of these chromatins show features not seen in the absorption curves: the ellipticity of unsheared chromatin increases with temperature, while this increase is abolished or greatly reduced in the same chromatin after shearing. After its first thermal transition (prior to the helix-coil transition) the unsheared chromatin achieves the same ellipticity as sheared chromatin.

INTRODUCTION

Chromatin studies would be facilitated if chromatin could be obtained in a homogenous solution, and for this reason, most authors have been studying the functional and structural properties of chromatin by shearing it, either with a motor-driven homogenizer (1) or by sonication (2). However, it has been recently reported that shearing causes a dramatic change in template activity (3), loss of the repeating units structure (4) and a large alteration in chromatin conformation (5,6), as seen by circular dichroism and ethidium bromide intercalation (5). In order to further investigate the structural changes induced in chromatin by shearing, we have employed thermal denaturation. Thermal denaturation has been extensively used to study the helix-coil transition in DNA, and as a probe of the stability and structure of DNA (7), of complexes of polypeptides and protein with DNA (8,9) and for chromatin (10,11,12 13). In the majority of these studies the thermal transitions have been monitored by observing the increase in absorption accompanying the helix-to-coil transitions: but in few cases (8,12) dichroism (CD) spectroscopy has been utilized in order to detect changes prior to melting (14), which may reflect subtle changes in the asymmetry of the DNA helix, prior to and

during the denaturation of chromatin (15). By using these sensitive techniques the drastic effects of shearing in chromatin structure appear confirmed and further characterized.

MATERIALS AND METHODS

H615 and AF8 cells both temperature sensitive mutants of established cell lines were grown as previously described (16, 17), at the permissive temperature of 32°C. Chromatin was isolated from these cells as previously described (13,18). The viscous chromatin pellet was either resuspended gently with a few strokes of a Dounce homogenizer in 1 mM Tris-HCl pH8 (unsheared) or sonicated at 50W for 30 seconds (sheared). Circular dichroism was measured using a Jasco Model J-40 recording spectropolarimeter. The instrument was standardized as previously described (5,6,17). All optical measurements were performed using a fused quartz cell with a water jacket for temperature regulation. Changes in ellipticity and optical density, at fixed wavelength, were monitored simultaneously during the thermal denaturation study. Both ellipticity and absorption were measured on our modified J-40 spectropolarimeter equipped with both circulating bath and PG-UL thermostat. Temperature was increased at a rate between 1°C/min and 2°C/min. The temperature was monitored with a Bailey amplifying thermometer (BAT-4) using a thermocouple (Bailey IT-1) placed at the cell jacket exit (time constant 0.8 sec, Teflon insulation). This allows accurate recording of the temperature. During measurements, chromatins were suspended in 1mM Tris-HCl, pH8. The derivative plots of the ellipticity (or absorbance) dependence on temperature, were performed on a time-sharing computer CDC-6400 (19) (not shown). Calf thymus DNA was obtained commercially (Sigma Corporation).

RESULTS

The absorption (Fig.1) and CD thermal denaturation at 276 nm (Fig. 2) yields an identical average melting temperature (Tm) of 55°C as determined by the derivative plots (19) for pure calf thymus DNA in 1mM Tris-HCl, pH8. As previously shown (8,20), the main helix-coil transition is characterized by an increase of absorption, paralleled by a decrease in ellipticity at 276 nm. Fig. 1 also shows the hyper-chromicity effect at 260 nm for the "sheared" and "unsheared" chromatin from H615 mutant cells, grown at the permissive temperature of 32°C. Shearing induces a decrease in thermal stability (melting

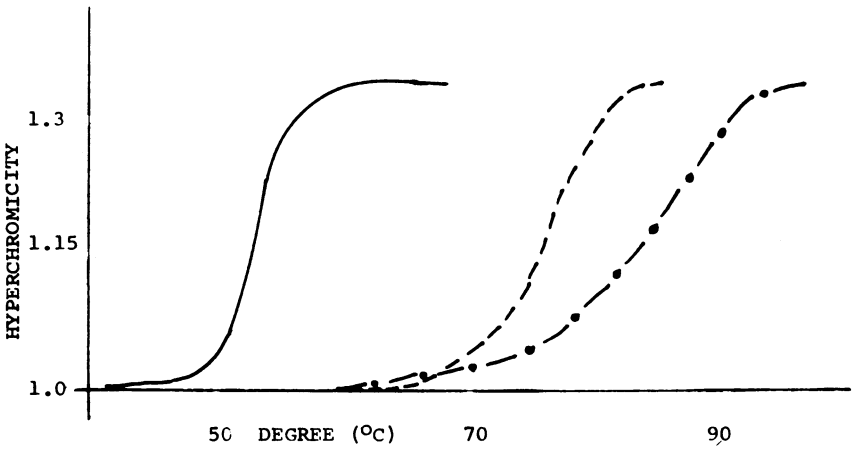

Fig. 1. Thermal denaturation profiles, by absorption changes at 260nm relative to absorption at room temperature (Hyperchromicity), of DNA from calf thymus (——), and of chromatin isolated from H615 cells grown at the permissive temperature of 32°C. The chromatin was either "unsheared" (—·—·) or "sheared" (– – – –). The solvent was 1 mM Tris-HCl, pH 8. Other details are given in Material and Methods.

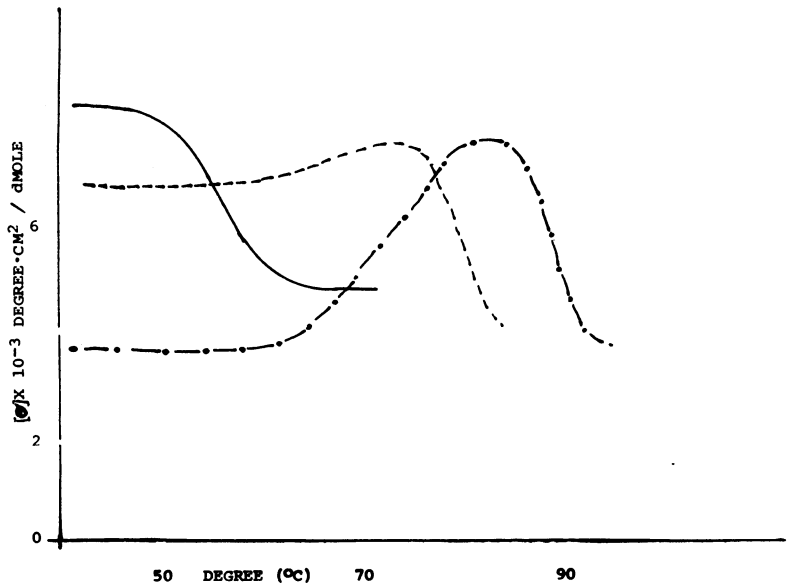

Fig. 2. Thermal denaturation profiles, by molar ellipticity changes at 276nm versus temperature, of DNA from calf thymus, (——) and of chromatin either "unsheared" (– – – –) or "sheared" (—·—·), isolated from H615 cells at the permissive temperature of 32°C. The solvent was 1mM Tris-HCl, pH 8.

temperature) of about 10°C. A similar decrease in the main helix-coil transition, after shearing, is shown by the temperature dependence of chromatin ellipticity, (Fig.2): specifically, the decrease in molar ellipticity, at 276 nm, starts at 74°C for the "sheared" and at 84°C for the "unsheared" chromatin. However, there are substantial differences between the extinction and the ellipticity curves. The ellipticity of the "unsheared" chromatin (Fig.2) exhibits an increase (maybe due to the superhelix to helix transition) at temperatures below the Tm of chromatin by about factor 2. On the other hand, the ellipticity curve of the "sheared" chromatin (from the same cell line), which has a substantially larger CD signal at 276 nm, as previously shown (5,6) at room temperature, lacks the increase in ellipticity that occurs before the main helix-coil transition. The same "unsheared" chromatin, in the presence of 0.50% SDS, presents a thermal denaturation profile similar to DNA calf thymus, with an identical lack or substantial reduction of premelting as for the "sheared" chromatin (Fig.3).

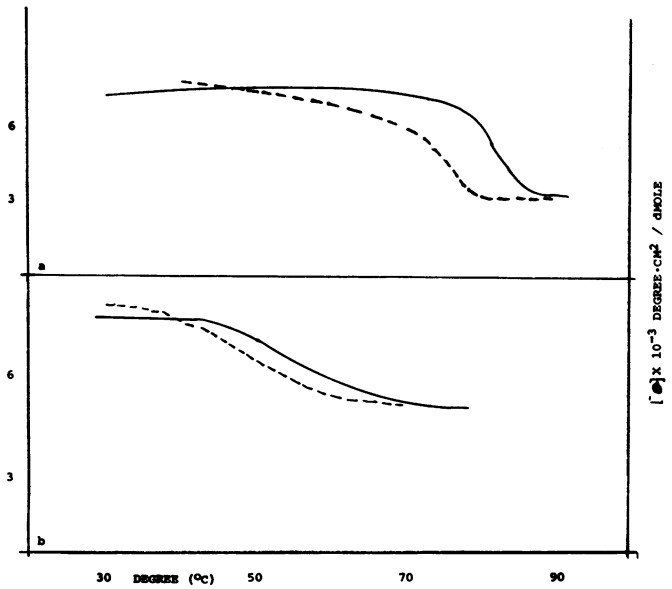

Fig. 3. Molar ellipticity changes at 276nm, during thermal denaturation (heating, ——) and renaturation (cooling, - - - -), of "sheared" chromatin from AF8 cells grown at the permissive temperature of 34°C, in 1mM Tris-HCl, pH 8 (upper panel) and in 0.5% Sodium Dodecyl Sulfate, 1mM Tris-HCl pH 8 (lower panel).

The differences between "sheared" chromatin and protein free DNA appear, moreover, evident during the renaturation, where the cooling process indicates different hysteresis cycles (Fig.3). Similarly, cooling of the "unsheared" chromatin in 1mM Tris-HCl, pH8 down to 30°C (Fig.4) completely renaturates

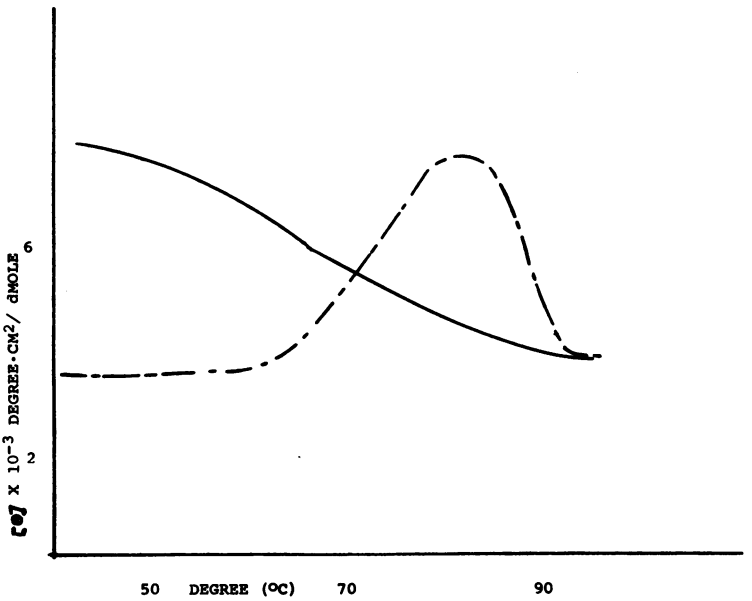

Fig. 4. Molar ellipticity changes at 276nm, during thermal denaturation (heating,----) and renaturation (cooling,——), of "unsheared" chromatin from H615 cells grown at the permissive temperature of 32°C, in 1mM Tris-HCl, pH 8.

the DNA double strand, but the ellipticity does not return to the original starting value, representative of a packed supercoiled DNA-structure in native "unsheared" chromatin (see Fig. 4). In fact, upon cooling down to room temperature, the "unsheared chromatin" ellipticity continuously increases until reaching the same maximum value as the "sheared" chromatin with an analgous renaturation process (see Fig. 3 and 4).

DISCUSSION

Most authors have been studying the functional and structural properties of chromatin by shearing it in order to have an homogenous solution. The artifacts introduced in this way are made evident in this communication by the large increase of CD signal at 276 nm and the decrease of thermal stability of

of chromatin after shearing. These findings parallel previous findings of the increase, after chromatin shearing, of positive ellipticity at 276 nm (5), ethidium bromide binding sites (5,6) and template activity (3) by a factor of 1.8, of the loss of repeating unit structure (4), and of differential light scattering (5,6). It must be emphasized that this chromatin disruption seems to exist independently of the methods of shearing explored (sonication at 50W from 20 seconds on, or motor-driven homogenizer for 30 seconds or more).

In conclusion this paper provides additional information on chromatin alterations induced by shearing and strongly suggests that "sheared" chromatin should not be used in studies of chromatin structure or function. Furthermore, the disappearance (or strong reduction) of "premelting" (i.e. increase in molar ellipticity) in chromatin combined with the decreased thermal stability of its main helix-coil transition after shearing, seems to suggest that a high degree of DNA super-packing (like in a superhelical configuration) exists in native "unsheared" chromatin, which is disrupted by shearing.

Similarly, while protein free DNA renatures, after cooling, the supercoil of DNA in native "unsheared" chromatin is irreversibly destroyed, evidently because protein-protein and protein-DNA interactions cannot occur with denaturated proteins (12).

ACKNOWLEDGEMENT

This work was supported by GRANTS number CA-18258 and CA-12923 from the National Cancer Institute.

REFERENCES

1. Clark, R.J. and Felsenfeld, G. (1971) Nat. New Biology 229, 101-104.
2. Chesterton, C., Courar, B.E. and Butterworth, P.H. (1974) Biochem. J. 143, 73-77.
3. De Pomerai, D.J., Chesterton, C.J. and Butterworth, P.H. (1974) Eur. J. Biochem. 4b, 471-482.
4. Noll, M., Thomas, T.O. and Kornberg, R.D. (1975) SCIENCE, 187, 1203-1207.
5. Nicolini, C., Baserga, R. and Kendall, F. (1976) SCIENCE, 192, 796-798.
6. Baserga, R. and Nicolini, C. Biophysica Biochemica Acta, Review on Cancer, (January 1976).
7. Marmur, T. and Doty, P. (1962) J. Mol. Biol. 5, 109-118.
8. Mandel, R. and Fasman, G. (1974) Biochem. Biophys. Res. Com.

59, 672-679.
9. Shih, T.Y. and Bonner, J. (1970) J. Mol. Biol. 50, 333-344.
10. Subirana, J.A. (1973) J. Mol. Biol. 74, 363-386.
11. Henson, P. and Walker, I.O. (1970) Eur. J. Biochem. 14, 345-350.
12. Wilhem, N., De Murcia, G., Campagne, M. and Daune, M. (1974) Nucleic Acids Research 45, 431-443.
13. Augenlicht, L., Nicolini, C. and Baserga, R. (1974) Biochemical Biophysical Research Com. 59, 920-926.
14. Gennis, R.B. and Cantor, C.R. (1972) J. Mol. Biol. 65, 381-398.
15. Henson, P. and Walker, I.O. (1970) Eur. J. Biochem. 16, 524-531.
16. Burstin, S.J., Meiss, H.K. and Basilico,C.J. (1974) J. Cell Physiol. 84, 397.
17. Ide, T. and Baserga, R. Biochemistry, in press.
18. Nicolini, C. Ng, S. and Baserga, R. (1975) Proc. Nat. Acad. Sci. 72, 2361-2366.
19. Milgram, E. (1975) Division of Biophysics Temple University, Internal Report 2/75 pp. 1-25.
20. Parodi, S., Nicolini, C., Sarma, S. and Farber, E. (1975) Biochem. Biophys. Acta 407, 174-190.