ISOLATION OF A RAT VENTRAL PROSTATE CHROMATIN FRACTION EXHIBITING A SINGULAR MELTING PROFILE

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SUMMARY

Rat ventral prostate chromatin, prepared by a gentle procedure in which marked shearing forces were avoided, was separated by sucrose gradient centrifugation into a quantitatively minor less dense (L) fraction, and 2 heavier components, (H_1) and (H_2). The (L) fraction, comprising 5-15 percent of the total chromatin DNA, had a unique melting profile, with a $Tm_{app.}$ of 59°, compared to 82.5°, 85° and 80.5° for total, (H_1) and (H_2) chromatin. (L) chromatin has a number of other properties expected of "euchromatin". This approach to the preparation of chromatin has been used successfully with other tissues such as liver.

INTRODUCTION

There is a great deal of evidence that in differentiated eukaryotic cells, only a small portion of the genome is actively transcribed (1,2). In addition, the regions used are species and tissue-specific (3,4). This fraction, termed "euchromatin", is distinguished from transcriptionally less active "heterochromatin" by a number of physical and chemical differences. For example, euchromatin has a higher

ratio of protein to DNA, lower Tm, greater template activity, is enriched in "unique" DNA sequences, and has a more open and extended configuration.

Efforts to separate eu and heterochromatin by exploiting these features usually include the use of intense shearing forces provided by homogenization (5), sonication (6) or sudden release of hydrostatic pressure (7). When vigorously sheared preparations are analyzed by sucrose gradient centrifugation, the distribution of fragmented chromatin is very heterogeneous (5). With Bio-Gel A-50m exclusion chromatography, only sheared preparations appear quantitatively in the eluate (8), and the initial "euchromatin" fraction can include more than 10 percent of the total chromatin A_{260} (7).

With these points in mind we asked the following simple question: if shearing forces used to prepare chromatin were strictly limited, could a quantitatively minor fraction of chromatin with the properties of "euchromatin" be isolated from total chromatin? We found that this was indeed so.

METHODS

Male Wistar or Long Evans rats, 3-5 months old were maintained on Purina chow and tap water ad libitum. Animals were killed by cervical dislocation and Triton X-100-washed prostate nuclei isolated as described (9). A method similar to that of Shaw and Huang (10, 11) was used to prepare chromatin.

Nuclei, resuspended in 75 mM NaCl-24mM EDTA solution, pH 8.0, were centrifuged at 7700 xg for 15 minutes. This

was repeated three times. The lysed residue was washed twice in 50 mM Tris solution, twice each in solutions of 10 mM Tris, 2 mM Tris, and finally in 0.4 mM Tris, all at pH 8 (2^{o}). During these washes, care was taken to gently detach pellets from the bottom of the centrifuge tubes, their vigorous dispersion strictly avoided, and low speed (7700xg, 8000 RPM, SS-34 rotor) centrifugation used to pellet the chromatin. The final pellet was stirred gently overnight in 0.4 mM Tris buffer with a small (5 mm length) stirring bar rotating at about 40 RPM, set off to one side of the vessel. This "minimally sheared" chromatin was suspended in the buffer with 5 strokes in a glass-on-glass homogenizer, and used immediately.

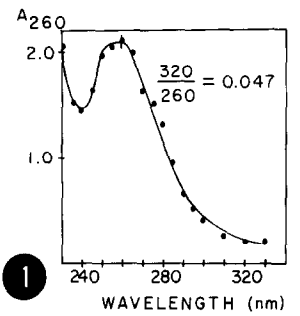
A 0.25 to 2.8 M sucrose gradient was prepared by layering 11 layers (0.4 ml, SW 50.1 rotor or 1.0 ml, SW 27 rotor) of the following sucrose solutions, containing 0.4 mM Tris, pH 8.0, 4^{o}: 2.8, 2.5, 2.2, 2.1, 1.75, 1.50, 1.25, 1.0, 0.75, 0.50, and 0.25M. The gradient was usually left in a refrigerator overnight before use. Chromatin was centrifuged for the times indicated, generally about 30 minutes at 64,000 xg. Tubes were punctured from the bottom, 20 or 30 drop samples collected and analyzed according to the protocol of the experiment.

Bio-Gel A-50m columns of 0.9 x 12 cm were eluted with 0.4 mM Tris, and 1.3 ml fractions collected with an LKB ultrorac 7000 fraction collector.

Chemicals were reagent-grade from several commercial sources, and biochemicals were obtained from Sigma and Bio-Rad.

RESULTS

Preparations of minimally sheared chromatin exhibited the characteristic chromatin absorption profile (Fig. 1.).



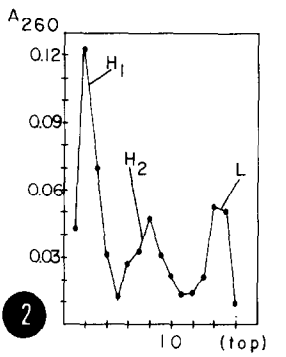


Fig. 1. Absorption spectrum of "minimally sheared" chromatin

Fig. 2. Sucrose gradient centrifugation of minimally sheared chromatin. Chromatin equal to 0.626 A_{260} was examined, with a recovery of 96 percent. (H_1) contained 46, (H_2) 18, and (L) 24 percent of the total A_{260}.

The low absorbance at 320 nm (320/260 = 0.047) is typical of highly purified chromatin that is neither contaminated with cytoplasmic proteins nor aggregated (12). When analyzed on 0.25 - 2.8 M sucrose density gradients, chromatin separated into 2 higher density fractions (H_1) and (H_2), which included 75-85 percent of the total A_{260}, and a quantitatively minor, lower density (L) component, accounting for the remaining absorbance (Fig. 2). Similar results were obtained with rat liver "minimally sheared" chromatin. If prostate chromatin was first subjected to 5 minutes of shearing in a Virtis homogenizer at "high" speed, (H) chromatin sedimented with the (L) fraction (not shown).

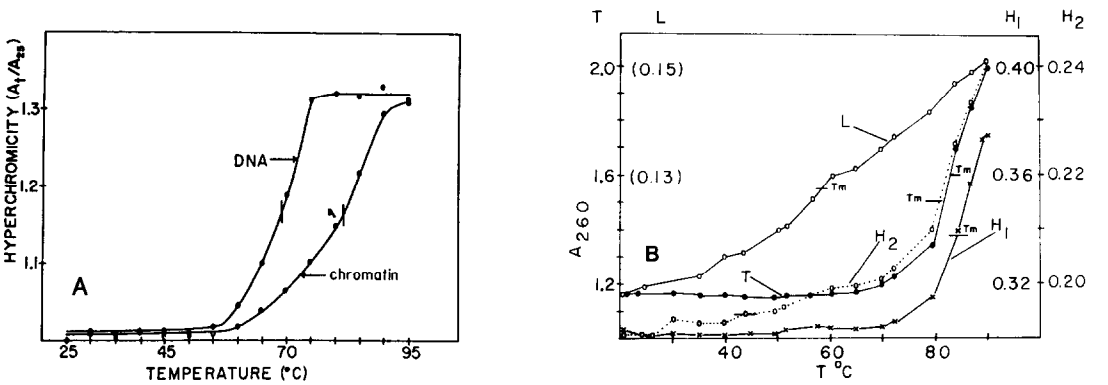


Fig. 3. Melting profile of prostate chromatin fractions. (3A) 0.35 A_{260} units of sheared chromatin and 0.22 units of deproteinized chromatin, suspended in 0.1 SSC were examined. Ordinate is absorbance at temperature t divided by A at t=25°. (3B) $Tm_{app.}$ of total, (H_1), (H_2) and (L) minimally-sheared chromatin. About 1.5 A_{260} units was centrifuged, fractions collected, and the Tm (midpoint of the hyperchromicity curves) from the peak fractions measured. T represents total chromatin.

The $Tm_{app.}$ of isolated (L) chromatin was compared with total (T), (H_1) and (H_2) fractions (Fig. 3). Unfractionated "minimally-sheared" chromatin had a Tm of 82.5°, and that of (H_1), (H_2), (L) and prostate DNA was 85 °, 80.5°, 59° and 69°. The reduced Tm of (L) chromatin probably reflects a more open and extended configuration.

Hyperchromicity of total, (H_1), (H_2) and (L) chromatin and prostate DNA was 70 (Fig. 3B), 24, 26, 41 and 33 percent (Fig. 3A) respectively. Melting curves of total and (H_1) chromatin and prostate DNA exhibited little hyperchromicity until 60-70° was reached, and sharp Tm profiles thereafter. Absorbance of (H_2) chromatin was biphasic, with gradually increasing absorbance until 70°, followed by a rapid rise. Absorbance of (L) chromatin increased continually from ambient temperature to 90°.

When unfractionated chromatin was analyzed by Bio-Gel A50m

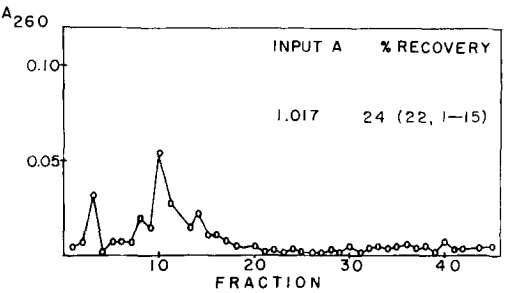


Fig. 4. Biogel A-50m chromatography of a representative chromatin preparation. Total recovery was 24 percent and 22 percent appeared in the first 15 fractions.

chromatography, 24 percent of the applied A_{260} was recovered in the eluate (Fig. 4). (L) fractions, isolated from a sucrose gradient and chromatographed, appeared quantitatively in the eluate (not shown). By this criteria, the 2 fractions are similar; further studies are in progress to try and establish their identity.

DISCUSSION

The (L) component has proven to exhibit some other interesting properties (manuscript submitted): (1). Since it migrated near the top of the sucrose density gradient, it must have a low density and/or extended configuration. This is confirmed by its appearance in the eluate from the Bio-Gel A-50 column (7). (2). (L) chromatin has a protein to DNA ratio of 7 to 1, compared with 0.6 and 0.8 for (H_1) and (H_2) fractions and 1.7 for total chromatin. (3). The absorbance maximum of (L) fraction is shifted to 270 nm, probably reflecting in part the greater content of protein. (4). The amount of DNA included in (L) chromatin was from 5 to 10 percent of

the total chromatin DNA. (5). Most trichloroacetic acid-precipitable counts originating from *in vivo* ^{14}C-uridine or orotic acid sedimented with the (L) fraction. (6). TCA-precipitable radioactivity derived from chromatin first incubated for RNA synthesis with E. coli RNA polymerase and then analyzed on sucrose gradients was largely associated with (L) chromatin. (7). The template activity (cpm/μg DNA, using E. coli RNA polymerase) of (L) chromatin was 5-10 times that of (H) material. (8). Radioactivity derived from *in vivo* administration of ^{3}H-testosterone, and almost certainly representing DHT (5α-androstan-17β-ol-3 one; 13,14) was associated equally with both peaks, but the specific radioactivity of the (L) fraction was 10 times (per unit DNA) that of (H) chromatin. (9). Greater residual endogenous RNA polymerase activity was found in pooled (L) chromatin.

By these criteria, (L) chromatin represents a form of "euchromatin". Since intense shearing was avoided during its preparation, gross contamination with less template-active heterochromatin should have been minimized. Whether (L) chromatin is functional *in situ* remains to be proven, although its association with *in vivo* labelled RNA observed both in sucrose gradients and Bio-Gel A-50m chromatography strongly suggests that it was (work in progress).

The chromatin fraction excluded from Bio-Gel A-50m, and the (L) fraction may be identical. Sucrose density gradient centrifugation (and possibly Bio-Gel A-50m chromatography) can be used to separate a template-active rat prostate fraction (L) of high purity from less active (H or hetero) chromatin, provided "minimally sheared" chromatin is prepared without vigorous disruption of its structure, and the attendant random scission of DNA.

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REFERENCES

1. Paul, J., Gilmour, R.S., J. (1968) J. Molec. Biol. 34, 305-316.
2. Bekhor, I., Kung, G.M., Bonner, J. (1969) J. Molec. Biol. 39, 351-364.
3. Paul, J., Current Topics in Devel. Biology 5, Academic Press p. 317-352.
4. Church, R.B., Brown, I.R., Nucleic Acid Hybridization in the Study of Cell Differentiation, ed. H. Urspring, Springer-Verlag, (1972), p. 11-24.
5. Chalkley, R., Jensen, R.H. (1968) Biochemistry 12, 4380-4387.
6. Frenster, J.H., Allfrey, V.G., Mirsky, A.E. (1963) Proc. Nat. Acad. Sci. U.S. 50, 1026-1032.
7. Janowski, M., Delill, S.N., McCarthy, B.J. (1972) in Gene Transcription in Reproductive Tissue, p. 112-129 Karolinska Institutet, Stockholm.
8. Vanden Broek, M.W.J., Hooden, L.D., Sevall, J.S., Bonner, J. (1973) Biochemistry 12, 229-236.
9. Anderson, K.M., Lee, F.H., Miyai, K. (1970) Exptl. Cell Res. 61, 371-378.
10. Shaw, J.M.J., Huang, R.C.C. (1970) Biochemistry 9 4530-4541.
11. Couch, R.M., Anderson, K.M. (1973) Biochemistry 12 3114-3121.
12. Bonner, J., Chalkley, G.R., Dahmus, M., Fambrough, D., Fujimura, F., Huang, R.C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B., Widholm, J. Methods in Enzymology. 12B (1968) p. 3-84.
13. Anderson, K.M., Liao, S., (1968) Nature 219 277-279.
14. Fang, S., Anderson, K.M., Liao, S., J.B.C. 244 6584-6595 (1969).