DEGRADATION OF CHROMOSOMAL PROTEINS DURING DISSOCIATION AND RECONSTITUTION OF CHROMATIN

Chi-Bom Chae and Donald B. Carter

Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27514

Received February 22,1974

SUMMARY. Histones and nonhistone chromosomal proteins are degraded when chromatin is exposed to 2 M NaCl-5 M urea (pH 6-8) which has been most often used for dissociation and reconstitution of chromatin. Histones and nonhistone proteins are also degraded in 5 M urea (pH 6-8).

Isolated chromatin mediates the synthesis of tissue-specific RNA's in the presence of RNA polymerase (1-4). Recently several reports have appeared on the dissociation and reconstitution of chromatin (5-11), and the most important criterion for correct reconstitution of chromatin has been the transcription of RNA's homologous to those transcribed from native chromatin (5-9). Most of the results with reconstituted chromatin were obtained with chromatin which was dissociated in 2 M NaCl-5 M urea, pH 6-8.3 and reconstituted by dialysis against decreasing concentrations of NaCl in the presence of 5 M urea. Others also have dissociated chromatin in 2 M NaCl-5 M urea in order to fractionate nonhistone proteins (7,9-13).

In this report we present evidence that chromosomal proteins are extensively degraded in 2 M NaCl-5 M urea at pH 6-8 and in 5 M urea at pH 6-8.

METHODS

Preparation of chromatin: Chromatin was prepared from pure rat liver nuclei as described before (14) and sheared at 20 volts for 90 seconds in a VirTis homogenizer.

Dissociation of chromatin: Chromatin was dissociated in freshly prepared, 2 M NaCl-5 M urea or 5 M urea by stirring slowly at 4°C for 3 hours, and the chromatin solution was centrifuged in a Beckman No. 40 rotor at 35,000 rpm at 4°C for 15-16 hours. The urea used in this study was from Schwartz/Mann (deionized ultra pure grade).

Copyright © 1974 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Sodium dodecyl sulfate gel electrophoresis: The supernatant obtained from chromatin dissociated in 2 M NaCl-5 M urea was dialyzed against cold 50 mM $NaHSO_3$, which is an inhibitor of chromatin-bound protease (15), for 3 hours and then against 1% sodium dodecyl sulfate-0.01 M phosphate (pH 7)-0.1% β-mercaptoethanol. However, the supernatant obtained from chromatin dissociated in 5 M urea was mixed with 0.1 vol. of 10% sodium dodecyl sulfate and dialyzed against the 1% sodium dodecyl sulfate mixture. The pellet containing DNA was dissolved in 1 ml of the 1% sodium dodecyl sulfate mixture with vigorous stirring.

The samples in sodium dodecyl sulfate were applied to 2.5% stacking gel (0.6 x 0.5 cm) (16) on top of 7.5% acrylamide separating gel (0.6 x 10 cm) in 0.1% sodium dodecyl sulfate, 2.5 M urea, 5 mM EDTA, and 0.1 M sodium phosphate, pH 7 (17) and electrophoresed as described before (14). The gels were stained with Coomassie Blue.

Electrophoresis of histones: For isolation of histones the chromatin dissociated for 20 hours at 4°C, but not centrifuged, was dialyzed against cold 50 mM $NaHSO_3$ and extracted with cold 0.4 N H_2SO_4 for 30 min. The supernatant obtained after centrifugation of the chromatin at 10,000 xg for 10 min was dialyzed against cold 7 M urea-0.9 N acetic acid-0.1% β-mercaptoethanol overnight. The histones were separated by acid-urea polyacrylamide gel (15% in 2.5 M urea) electrophoresis (18).

RESULTS AND DISCUSSION

Table 1 summarizes the composition of solutions used for dissociation of chromatin (mixtures of salt and urea) and for washing of reconstituted chromatin (5 M urea). There have been several reports on the partial degradation of histones when thymus chromatin is exposed to 5 M urea-2 M NaCl-10 mM Tris, pH 8 (8,19), but as far as we can determine there has been no report which demonstrates that chromosomal nonhistone proteins are or are not degraded during the exposure of chromatin in the solutions listed in Table 1. When chromatin is dissociated for preparation of nonhistone proteins (9-13) or reconstitution

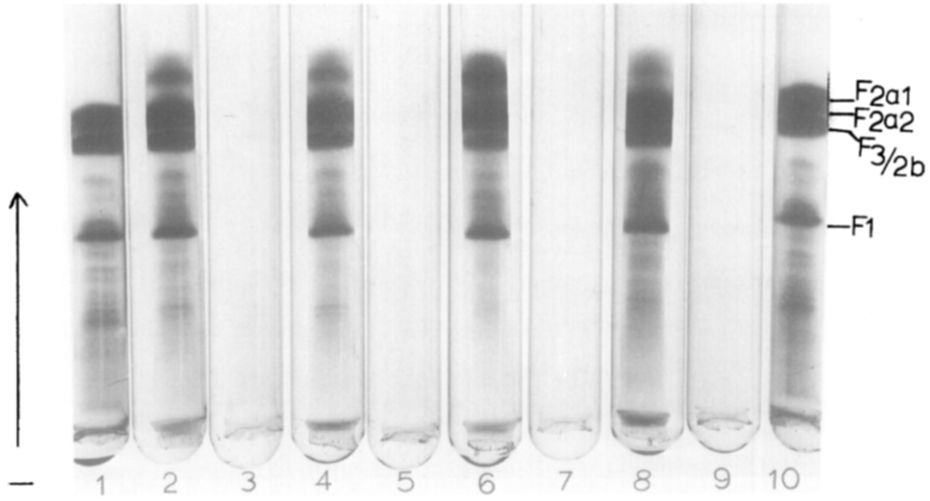

Figure 1. Sodium dodecyl sulfate gel electrophoresis of chromatin dissociated in 2 M NaCl-5 M urea. Control chromatin (undissociated) (1); 2 M NaCl-5 M urea-50 mM acetate, pH 6, supernatant (2) and pellet (3); 2 M NaCl-5 M urea-50 mM phosphate, pH 7, supernatant (4) and pellet (5); 2 M NaCl-5 M urea-10 mM Tris, pH 8, supernatant (6) and pellet (7); 2 M guanidine·HCl-5 M urea, pH 6.5, supernatant (8) and pellet (9); control chromatin (10). Experimental details are described in Methods.

of chromatin (5-11), chromatin is exposed to a mixture of NaCl or guanidine·HCl and urea between 16 hours and several days at 4°C, and we found that under such condition considerable amounts of nonhistone proteins and histones are degraded. For example, when rat liver chromatin is dissociated in 2 M NaCl-5 M urea at pH 6, 7, and 8 and in 2 M guanidine·HCl-5 M urea as described in Methods, it was found that the supernatant contains a considerable amount of low molecular weight proteins which are not seen in native chromatin (Figure 1). In the case of control chromatin, which was directly dissolved in the 1% sodium dodecyl sulfate mixture, F2a1 histone is the smallest protein in liver chromatin. It appears from the electrophoretic patterns that mainly high molecular weight nonhistone proteins are decreased in amount in the chromatin dissociated in the mixture of salt and urea while polypeptides of molecular weights smaller than histone F2a1 appear in the electrophoretic patterns. The degradation is more extensive at pH 8 than at lower pHs. The same results were obtained with the chromatin dissociated in the same solutions used in Figure 1 but not centrifuged (not shown here).

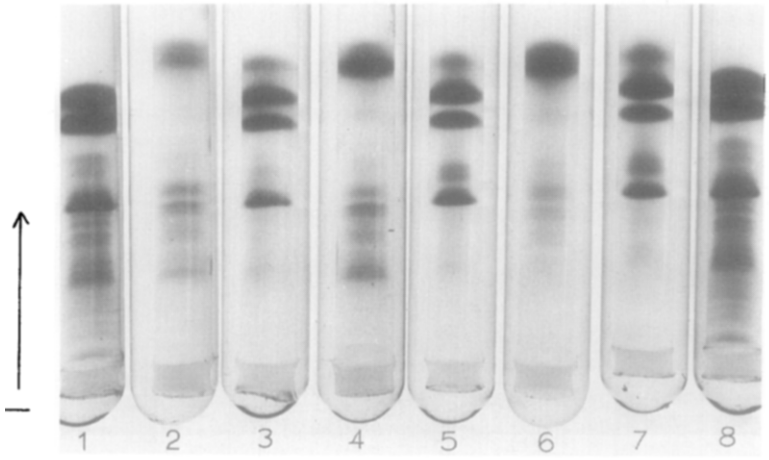

Figure 2. Sodium dodecyl sulfate gel electrophoresis of chromatin dissociated in 5 M urea. Control chromatin (1); 5 M urea-50 mM acetate, pH 6, supernatant (2) and pellet (3); 5 M urea-50 mM phosphate, pH 7, supernatant (4) and pellet (5); 5 M urea-10 mM Tris, pH 8, supernatant (6) and pellet (7); control chromatin (8). Experimental details are described in Methods. In this experiment chromatin was exposed to 6-7°C during 3 hour stirring before centrifugation.

High molecular weight nonhistone proteins are also decreased in quantity in the chromatin dissociated in 5 M urea at pH 6, 7, and 8 as shown in Figure 2. However, the degradation seems to be somewhat less at pH 6 and 7 than at pH 8. As also shown in Figure 2 most of nonhistone proteins, but not histones, are dissociated from chromatin by 5 M urea. Dissociation of nonhistone proteins from DNA by 4 M urea-0.1 M phosphate (pH 7) has also been reported (20).

Since histones are not well resolved in sodium dodecyl sulfate gel electrophoresis, we extracted histones by cold 0.4 N H_2SO_4 from the chromatin dissociated in 2 M NaCl-5 M urea and in 5 M urea at different pHs, and the histones were separated by acid-urea gel electrophoresis (18). As shown in Figure 3 histones from the chromatin dissociated in 2 M NaCl-5 M urea at pH 6 and 7 and in 2 M guanidine•HCl-5 M urea are relatively intact although small amounts of degradation products migrate before F2a1 histone, but histones are considerably degraded in 2 M NaCl-5 M urea at pH 8. On the other hand, considerable amounts of F1, F2b and F2a2 histones are degraded in 5 M urea at pH 6 and 7 but not as much in 5 M urea at pH 8. Histones of the chromatin dis-

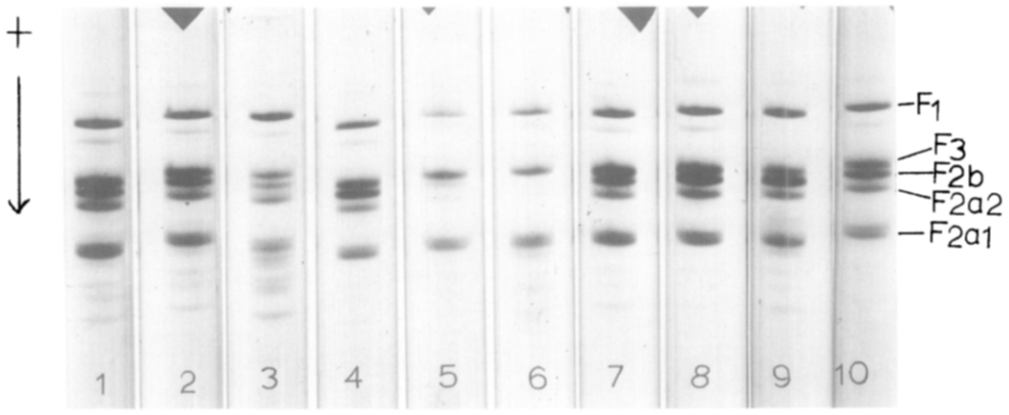

Figure 3. Acid-urea gel electrophoresis of histones extracted from chromatin dissociated in 2 M NaCl-5 M urea and in 5 M urea. (1) 2 M NaCl-5 M urea-50 mM acetate, pH 6; (2) 2 M NaCl-5 M urea-50 mM phosphate, pH 7; (3) 2 M NaCl-5 M urea-10 mM Tris, pH 8; (4) 2 M guanidine·HCl-5 M urea, pH 6.5; (5) 5 M urea-50 mM acetate, pH 6; (6) 5 M urea-50 mM phosphate, pH 7, (7) 5 M urea-10 mM Tris, pH 8; (8) 2 M NaCl; (9) 2 M NaCl-10 mM Tris, pH 8; (10) control chromatin.

sociated in 2 M NaCl are intact but a significant amount of F2a1 histone is degraded in 2 M NaCl-10 mM Tris, pH 8.

The results described here suggest that there could have been extensive degradation of nonhistone proteins and histones during the reported reconstitution of chromatins which are known to have protease activity such as liver and thymus chromatin (15,21). However, it is quite possible that chromatin isolated from other tissues or cultured cell lines listed in Table 1 may also contain protease. We have followed the reported reconstitution procedure with rat liver chromatin at pH 6 and 8, and as expected high molecular weight nonhistone proteins are depleted in the final reconstituted chromatin (not shown here). In some instances the reconstituted chromatin was washed with 5 M urea-10 mM Tris, pH 8.3 (10,11) which removes most of the nonhistone proteins from DNA in the case of liver chromatin (Figure 2). We found that the urea-extracted chromatin of liver and thymus is usually precipitated in dilute buffer as one expects for a histone-DNA complex having histone/DNA ratio of

Table 1

Reported Dissociation Conditions for Chromatin and Tissues Studied

Dissociation Solution	Tissues	Reference
2 M NaCl - 5 M urea-50 mM acetate, pH 6	liver, thymus	8
2 M NaCl - 5 M urea-1 mM phosphate, pH 7	liver, thymus, erythrocytes	12
2 M NaCl - 5 M urea-10 mM Tris, pH 8 - 8.3	pea codyledon, liver, thymus, kidney, HeLa cells, Wl-38 fibroblasts	5,7, 9-11,13
2 M guanidine·HCl - 5 M urea	chick embryo	6
5 M urea - 10 mM Tris, pH 8.3	Wl-38 fibroblasts, HeLa cells	10,11

1-1.2 (22) as in most chromatin preparations (20). It is very likely that substantial amounts of nonhistone proteins are also dissociated from chromatin isolated from other tissues and cell lines.

In summary, although previously reported conditions for reconstitution of chromatin have been reported to yield chromatin which, when used as templates in transcription experiments, yielded RNA's similar to those transcribed from native chromatin, the present experiments raise questions about the integrity of the chromosomal proteins in these reconstituted chromatins and thus create doubts about the validity of the interpretations of the transcription experiments, which were done under the conditions which only permit the detection of RNA transcripts from repetitive sequences of DNA (23).

ACKNOWLEDGEMENT

This work was supported by grants from the University of North Carolina (VF 336) and the American Cancer Society (IN15-0) and USPHS General Research Support Award (5 SO1-FR-05406).

REFERENCES

1. Paul, J. and Gilmour, R.S. (1968) J. Mol. Biol. 34, 305-316.
2. Smith, K.D., Church, R.B. and McCarthy, B.J. (1969) Biochemistry, 8, 4271 4277.
3. Axel, R., Cedar, H. and Felsenfeld, G. (1973) Proc. Nat. Acad. Sci. US, 70, 2029-2032.

4. Gilmour, R.S. and Paul, J. (1973) Proc. Nat. Acad. Sci. US 70, 3440-3442.
5. Bekhor, I., Kung, G.M. and Bonner, J. (1969) J. Mol. Biol. 39, 351-364.
6. Huang, R.C.C., and Huang, P.C. (1969) J. Mol. Biol. 39, 365-378.
7. Gilmour, R.S. and Paul, J. (1969) J. Mol. Biol. 40, 137-139.
8. Spelsberg, T.C., Hnilica, L.S. and Ansevin, A.T. (1971) Biochim. Biophys. Acta, 228, 550-562.
9. Gilmour, R.S. and Paul, J. (1970) FEBS Lett, 9, 242-244.
10. Stein, G., Chaudhuri, S. and Baserga, R. (1972) J. Biol. Chem. 247, 3918-3922.
11. Stein, G. and Farber, J. (1972) Proc. Nat. Acad. Sci. US 69, 2918-2921.
12. MacGillivray, A.J., Cameron, A., Krauze, R.J., Rickwood, D. and Paul, J. (1972) Biochim. Biophys. Acta, 277, 384-402.
13. Richter, K.H. and Sekeris, C.E. (1972) Arch. Biochem. Biophys. 148 44-53.
14. Smith, M.C. and Chae, C-B. (1973) Biochim. Biophys. Acta, 317, 10-19.
15. Bartley, J. and Chalkley, R. (1970) J. Biol. Chem. 245, 4286-4292.
16. Waehneldt, T.V. and Mandel, P. (1970) FEBS Lett, 9, 209-212.
17. Bhorjee, J.S. and Pederson, T. (1972) Proc. Nat. Acad. Sci. US 69, 3345-3349.
18. Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346.
19. Kleiman, L. and Huang, R-C.C. (1972) J. Mol. Biol. 64, 1-8.
20. Hnilica, L.S. (1972), The Structure and Biological Function of Histones. CRC Press, Cleveland, Ohio.
21. Garrels, J.I., Elgin, S.C.R. and Bonner, J. (1972) Biochem. Biophys. Res. Commun. 46, 545-551.
22. Shih, T.Y. and Bonner, J. (1970) J. Mol. Biol. 48, 469-487.
23. Melli, M. and Bishop, J.O. (1969) J. Mol. Biol. 40, 117-136.