THE PRESENCE OF F3-F2a1 DIMERS AND F1 OLIGOMERS IN CHROMATIN

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Received March 17,1975

SUMMARY

The oligomeric structure of histones in nuclei and chromatin has been studied by crosslinking nuclei and chromatin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Crosslinked histones were detected as new high molecular weight components on SDS gels, and the protomers of the crosslinked histones were identified by their characteristic ^{125}I-fingerprints. The results show that a considerable portion of histones F3 and F2a1 exist in nuclei and chromatin as an F3-F2a1 dimer. Evidence is presented that histone F1 probably exists in chromatin as large oligomers.

Introduction

The molecular structure of chromatin has been partially illuminated by recent studies of its ultrastructure (1), susceptibility to nucleases (2-4), and selective extractibility of histones (5). These studies have been interpreted to indicate that chromatin consists of a more or less repetitive sequence of simpler histone containing units. Other investigators have found that the histones, when free of DNA, form stoichiometric complexes (6-11) and it has been suggested that these complexes are important determinants of chromatin structure (8,10). However, direct evidence for specific interactions among histones in chromatin is lacking.

One approach to this problem is to crosslink neighboring proteins in a complex, then isolate and identify the crosslinked products. This approach has been successfully applied to ribosomes and some oligomeric proteins (12,13). We applied this technique to chromatin and nuclei using a carbodiimide to crosslink neighboring histones. In this paper we report that a considerable portion of histones F3 and F2a1 exist in chromatin

as F3-F2a1 dimers. Evidence is also presented that most of F1 in chromatin is present as homooligomers.

METHODS

Nuclei were prepared from fresh rat liver by the method of Clark and Felsenfeld (14), or by the method of Hewish and Burgoyne (2). Both methods of preparation gave similar results in these experiments. Nuclei from the above preparations were washed into 0.2 mM EDTA pH 7.0 and chromatin was prepared by the method of Clark and Felsenfeld (14).

Reaction mixtures for crosslinking routinely contained nuclei (0.3-1.0 mg DNA/ml) or chromatin (0.1-1.0 mg DNA/ml), sodium phosphate (5 mM, pH 7) or triethanolamine (5 mM, pH 8) buffer, 0.2 mM EDTA and 3 mM 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide hydrochloride (Pierce Chemical Co.). Mixtures were incubated for one hour at 20°C. Reactions were stopped by the addition of SDS gel buffer or by dialysis vs. 1 mM sodium phosphate pH 7.

Samples were analyzed on SDS gels according to Laemmli (15) except that 18% acrylamide and 0.09% bis-acrylamide were used for the resolving gel.

Gels were stained with 0.1% Coomassie brilliant blue R250 in 40% ethanol, 10% acetic acid and destained in the same solution lacking dye. Dye and protein was extracted from the gels by the method of Bray and Brownlee (16) into 0.1% SDS. The amount of dye eluted was determined by its absorbance at 550 nm. Control experiments showed that the amount of dye extracted is proportional to the amount of protein loaded onto the gels. Assuming that the crosslinked histones bind dye as efficiently as free histones, one can then roughly quantitate the fraction of histone present in the crosslinked bands.

Material eluted from gel bands was then iodinated with ^{125}I (Amersham-Searle Na ^{125}I carrier free) and mixed with total calf thymus histone labeled with non-radioactive iodine. The mixture was digested with TPCK-trypsin (Worthington Biochemical) according to Bray and Brownlee (16) and fingerprinted according to Weintraub and Van Lente (4). ^{125}I-containing spots were located by autoradiography with Kodak RP Royal X-Omat film. Non-radioactive iodine containing spots were located with the stain of Gmelin and Virtanen (17). The staining pattern served

as an internal standard for the fingerprinting procedure. Control experiments showed that the spots of the radioactive fingerprint of rat liver histone fractions coincided with spots of the non-radioactive fingerprint of total calf thymus histone.

RESULTS

A typical pattern for rat liver chromatin on SDS gels is shown in Figure 1a. After crosslinking with 3 mM carbodiimide for one hour at pH 7, the pattern seen in Figure 1b is obtained. Under these conditions the histones are not completely crosslinked but a major new band (called α) appears at an R_f of 0.65 (relative to F2a1). Material also appears at the top of the resolving gel (called top). (There is also a crosslinked band between F1a and F1b which will not be discussed here.)

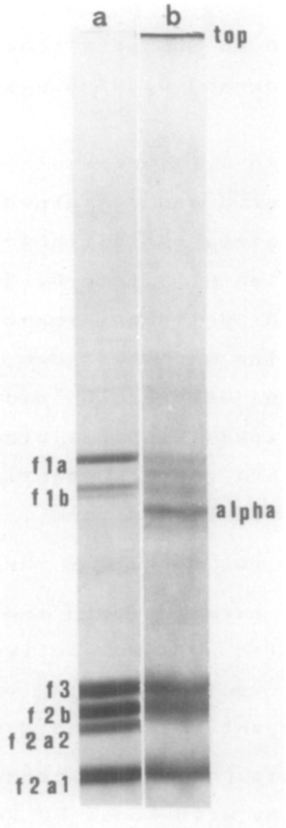

Figure 1. SDS gel electrophoresis of rat liver chromatin before (a) and after (b) reaction with carbodiimide.

Four pieces of evidence indicate that α is formed from neighboring molecules on the same chromatin particle rather than different particles. Firstly, α is formed in rat liver nuclei as well as rat liver chromatin. Secondly, α is formed in chromatin from chick erythrocytes as well as rat liver. Thirdly, the rate of formation of α is independent of chromatin concentrations from 0.1-1.0 mg DNA/ml). Finally, in 0.14 M NaCl, conditions which lead to precipitation of chromatin, the formation of α is not increased and the overall gel pattern is identical to that obtained in low salt.

A comparison of the ^{125}I fingerprints of α with those of the five known histone fractions showed that α contains F3 and F2a1 (Figure 2a-d). Four major and at least three minor α spots coincided with spots from F3 and another four major and two minor spots of α coincided with spots from F2a1. Spots from F2a2, F2b, or F1 were not found in the fingerprints of α.

The R_f of α coincides with that expected for a histone of approximately 26,000 daltons (Figure 3). The sum of the molecular weights of F3 and F2a1 is 26,6000 daltons. These two pieces of evidence show that α is a F3-F2a1 dimer.

Amount of α.

The amount of α appearing relative to the amount of F3 and F2a1 disappearing was calculated by extracting the dye from the gel bands. Under the conditions of Figure 1 about half the small histones had been crosslinked. Assuming α reacts with dye to the same extent as F3 and F2a1, the amount of α appearing could account for 55-95% of the F3 and F2a1 reacted. Therefore, under conditions in which about half the histones were crosslinked, most and perhaps all of the crosslinked F3 and F2a1 could be found in α.

Under more extensive crosslinking conditions, the amount of α did not increase as F3 and F2a1 disappeared. This result may indicate that some F3 and F2a1 in chromatin cannot be crosslinked by carbodiimide, i.e. that there is heterogeneity in the associations of F3 and F2a1. It is also possible that α is being further crosslinked to larger materials at the same time as α is being formed from free F3 and F2a1; however no higher molecular weight bands appear in the resolving gel after more extensive crosslinking.

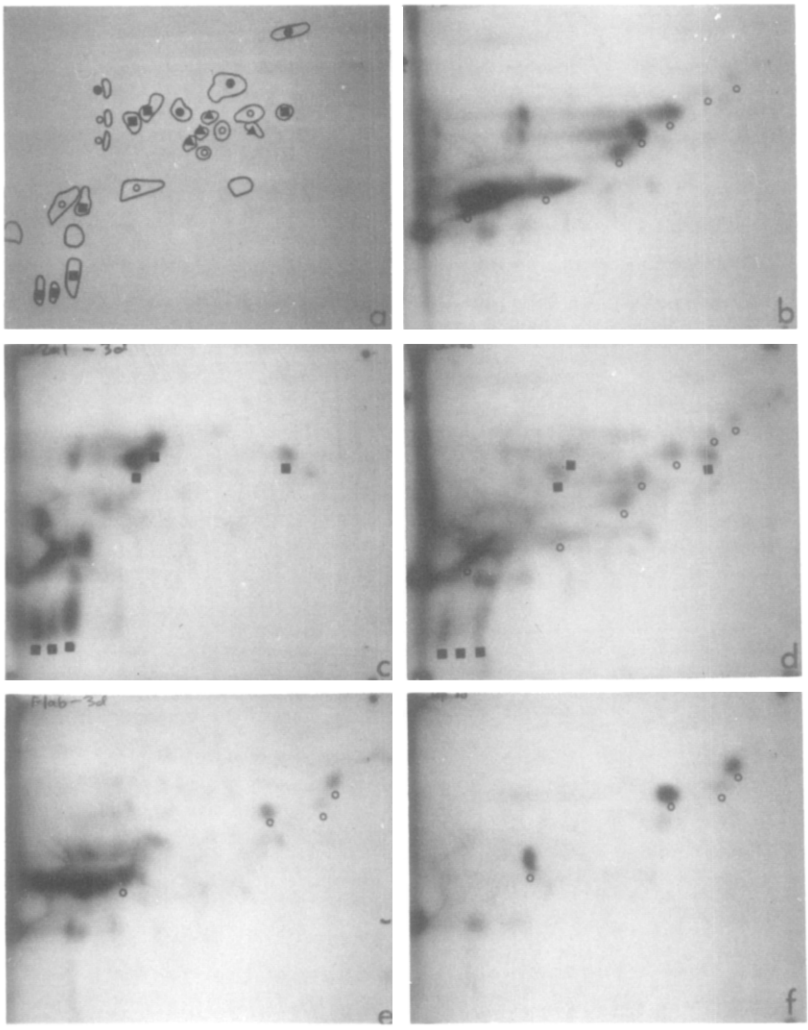

Figure 2. ^{125}I-fingerprint analysis of rat liver histones and crosslinked materials. (a) A composite of the major ^{125}I-spots from the four small histones. ■ F2a1, 0 F3, ● F2a2, ▲ F2b. (b-f) ^{125}I-fingerprints of F3 (b), F2a1 (c), α (d), F1 (e), and "top" (f). Open circles in (e) and (f) indicate ^{125}I-spots from F1.

Samples were spotted in the upper left hand corner and chromatographed towards the bottom. Electrophoresis is in the horizontal direction with the cathode at the right.

Identity of Top.

^{125}I fingerprints of the material at the top of the resolving gel shows spots from F1 only (Figure 2e,f). No spots from F2a1,

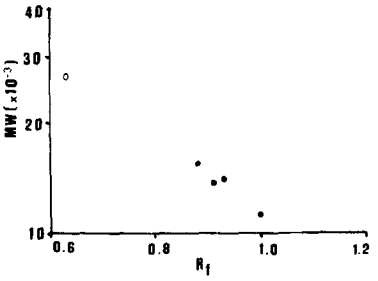

Figure 3. R_f's of α and the four small histones vs. molecular weight. The molecular weight of α is calculated to be the sum of F2a1 and F3 (26,600 daltons). With increasing R_f, the proteins are α, histones F3, F2b, F2a2 and F2a1.

F2a2, F2b or F3 are visible. Since DNase and RNase treatment do not affect this result, this material is probably primarily large oligomers of F1. Data from dye extraction studies indicate that this material can account for all the F1 removed from the monomer bands.

DISCUSSION

This study shows that carbodiimide crosslinks F3 and F2a1 to each other in chromatin and nuclei. The nature of the crosslink has not as yet been determined; however, two properties of the crosslink are evident from the data. First, the reaction conditions are such as to favor the formation of amide bonds (18), a fact which suggests that the crosslink may be between a carboxyl group on one histone and a lysine on the other. Second, because the α fingerprint is almost an exact superposition of the F3 and F2a1 fingerprints, the overall modification of the histones has been small and probably only one or very few crosslink exists between F3 and F2a1.

Kornberg and Thomas (8), and D'Anna and Isenberg (9) have found that F3 and F2a1 in solution, free of DNA, form tetramers. The presence of the F3-F2a1 dimer in chromatin is consistent with their suggestions that the interactions among histones in solution is also found in chromatin (8-10). However, our studies on the relative reactivity of F3 and F2a1 oligomers free in solution and of these histones in chromatin indicate that the histones in these two states may have quite different conformations (Bonner, unpublished observations).

The crosslinking of F1 in chromatin to form oligomers and the absence of other histones in this material suggest that F1 may exist in chromatin as long homopolymers, not intimately associated with the complexes formed among the other histones and DNA. This result is supported by that of Ölins and Wright (19) who found material from glutaraldehyde-crosslinked chick erythrocyte nuclei which upon amino acid analysis seemed to be primarily oligomers of histone F2c.

Using formaldehyde crosslinking, and fingerprint analysis of the crosslinked material, Weintraub (personal communication) has found F2b-F2a2 and F2b-F2a1 pairs in chromatin. It is striking that the histones crosslinked by carbodiimide and formaldehyde are the same three pairs of strong histone interactions reported by D'Anna and Isenberg (9).

These studies present direct evidence that at least some of the associations of histones in chromatin may be the same as the associations of the histones in solution.

Histone Nomenclature. F3=III=ARE, F2a1=IV=GRK, F2b=IIb2=KAS F2a2=IIb1=LAK, F1=I=KAP

REFERENCES

1. Ölins, A. L., and Ölins, D. E. (1974) Science 183, 330-332.
2. Hewish, D. R., and Burgoyne, L. A. (1973) Biochem. Biophys. Res. Commun. 52, 504-510.
3. Axel, R., Melchior, W., Jr., Sollner-Webb, B., and Felsenfeld, G. (1974) Proc. Nat. Acad. Sci. USA 71, 4101-4105.
4. Weintraub, H., and Van Lente, F. (1974) Proc. Nat. Acad. Sci. USA. 71, 4249-4253.
5. Varshavsky, A. J., and Georgiev, G. P. (1972) Biochem. Biophys. Acta 281, 669-674.
6. Skandrani, E., Mizon, J., Sautiere, P., and Biserte, G. (1972) Biochemie 54, 1267-1272.
7. Kelley, R. I. (1973) Biochem. Biophys. Res. Commun. 54, 1588-1594.
8. Kornberg, R. D., and Thomas, J. O. (1974) Science 184, 865-868.
9. D'Anna, J.A., Jr., and Isenberg, I. (1974) Biochemistry 13, 4992-4997.
10. D'Anna, J.A., Jr., and Isenberg, I. (1974) Biochemistry 13, 2098-2104.
11. D'Anna, J.A., Jr., and Isenberg, I. (1973) Biochemistry 12, 1035-1043.
12. Sun, T-T., Bollen, A., Kahan, L., and Traut, R. R. (1974) Biochemistry 13, 2334-2340.
13. Carpenter, F. H., and Harrington, K. T. (1972) Journal of Biol. Chem. 247, 5580-5586.
14. Clark, R. J., and Felsenfeld, G. (1971) Nature New Biology 229, 101-105.
15. Laemmli, U. K. (1970) Nature 227, 680-685.
16. Bray, D., and Brownlee, S. M. (1973) Analytical Biochemistry 55, 213-221.
17. Gmelin, R., and Virtanen, A. I. (1959) Acta Chem. Scand. 13, 1469-1470.
18. Carraway, K. L., and Koshland, D. E., Jr., in Methods in Enzymology XXV, ed. C.H.W. Hirs and S.N. Timasheff, Academic Press (1972) p. 616-622.
19. Ölins, D. E., and Wright, E. B. (1973) Journal of Cell Biology 59, 304-317.