Isolation of a 167 basepair chromatosome containing a partially digested histone H5

Pere Puigdomènech, Matilde José, Adolfo Ruiz-Carrillo+ and Colyn Crane-Robinson*

Unidid de Biofisica y Biologia Molecular, Instituto de Biologia de Barcelona, CSIC, Edificio 'Juan de la Cierva', c/o Jorge Girona Salgado, s/n, Barcelona 34, Spain, +Centre de Recherche, Hôtel-Dieu de Quebec, Quebec, Canada and **Biophysics Laboratories, Portsmouth Polytechnic, St Michael's Building, White Swan Road, Portsmouth, Hants PO1 2DT, England*

Received 21 December 1982

A test has been made of the proposal that protection of the 167 basepair DNA length in the 'chromatosome' is due only to the central globular domain of the lysine-rich histones. Chicken erythrocyte chromatin was treated with trypsin to leave only the limit peptide from histones H1 and H5. Nucleosome monomers were then isolated on sucrose gradients following micrococcal nuclease digestion and were found to contain the 167 basepair DNA band as in intact chromatin. The presence of the limit peptide from H5 on the monomers was confirmed using an antibody to H5. It is concluded that the trypsin-susceptible domains of the lysine-rich histones are not involved in the protection of the 2-turn 167 basepair length of DNA in the nucleosome.

Histone H1 *Chromatin* *Chromatosome* *Histone H5*

1. INTRODUCTION

A range of evidence [1–6] indicates that the lysine-rich histones H1 and H5 are involved in maintaining the higher-order structure of chromatin. Studies of these histones in free solution show them to consist of 3 domains: a central folded domain and two flanking disordered domains [7–12]. Nuclease digestion has demonstrated [4] that histone H1 is responsible for protecting the ~20 basepairs (bp) of DNA that are present in the chromatosome [13] but absent in the core particle. We have subsequently shown that this protection can be re-established in H1-depleted chromatin by reconstitution of only the central folded domain and therefore proposed that this domain of H1 and H5 closes the second full turn of DNA in the nucleosome and acts to locate the H1 molecule [14]. However, it could be argued that in reconstitution with the globular domain alone this fragment locates itself in the most negatively charged region of the chromatin and the binding site observed is not necessarily the natural one. The experiment reported here seeks to circumvent this problem by treating chromatin with trypsin so as to leave only the globular domain of H1 and H5 and subsequently digest with nuclease to see whether the 167 bp length is still protected.

2. MATERIALS AND METHODS

Nuclei from chicken erythrocytes were prepared essentially as in [15] and digested with micrococcal nuclease (Worthington) in 110 mM KCl, 30 mM NaCl, 0.2 mM $MgCl_2$, 0.4 mM PMSF, 1 mM $CaCl_2$, 10 mM Tris–HCl (pH 7.4) at 60 units of enzyme/mg DNA and 2 mg DNA/ml for 30 min at 4°C. The reaction was stopped by the addition of EDTA to 2.5 mM. Soluble chromatin was obtained by resuspending the nuclei in 40 mM KCl, 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4) followed by centrifugation at 10000 rev./min for 10 min. The yield of chromatin in these conditions is ~80% of the total in the nuclei.

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

The soluble chromatin was then digested with trypsin (Sigma) at a ratio of 1 μg enzyme/200 μg DNA, using 1–2 mg DNA/ml at 4°C. Samples to be analysed by electrophoresis were either precipitated with 20% trichloroacetic acid or extracted with 5% perchloric acid (PCA) [10] to extract histones H1 and H5 and their peptides. Electrophoresis buffer contained 0.4 mM PMSF and 1 μg TLCK/ml (tosyl L-lysine chloromethyl ketone). Chromatin samples were treated with TLCK and soybean trypsin inhibitor prior to nuclease digestion.

To obtain chromatosomes from trypsin-treated chromatin, the latter was treated with micrococcal nuclease (100 units/mg DNA) at 37°C at, effectively, 0.6 mM $CaCl_2$. The reaction was stopped with EDTA at 2.5 mM. The samples were then centrifuged for 5 min in a microcentrifuge and loaded onto 5–20% linear sucrose gradients containing 40 mM KCl, 0.2 mM EDTA, 0.4 mM PMSF, 1 μg TLCK/ml, 10 mM Tris–HCl (pH 7.4). The gradients were run for 18 h at 40000 rev./min and 4°C in a Beckman SW 40 rotor. Gradient profiles were monitored with an ISCO cell and 1 ml fractions collected.

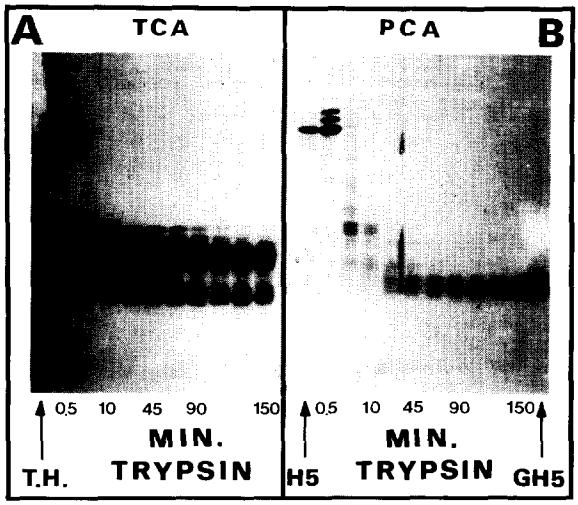

Fig.1. 18% polyacrylamide gel electrophoresis of proteins precipitated by 20% trichloroacetic acid (TCA) or extracted by 5% perchloric acid (PCA) from soluble chicken erythrocyte chromatin treated with trypsin for the time indicated. TH is a total histone control. H5 is a pure sample of histone H5 and GH5 is a pure sample of the limit peptide from histone H5 prepared from trypsin digestion of H5 in free solution.

Protein blotting was done essentially as in [16,17] by transferring the proteins to nitrocellulose filters (Millipore) by electrophoretic transfer. Filters were preincubated at 37°C in 0.15 M NaCl, 50 mM Tris–HCl, 0.05% Nonidet NP40, 3% Bovine serum albumin (Miles) (pH 7.4) then incubated in the same buffer with 20 μg/ml of anti-H5 antibodies raised in rabbits and purified by immunoabsorption [18]. The filters were developed with fluorescein-conjugated sheep anti-rabbit antibody (Gibco) at 1/50 dilution. The filter was washed with 1 M NaCl, 50 mM Tris–HCl, 0.2% sarkosyl (pH 7.4) and finally photographed with a yellow–green filter under UV (254 nm) light.

Protein samples were analysed in 18% polyacrylamide slab gels following the Laemmli technique [19]. DNA samples were analysed in 10% polyacrylamide gels run in Tris–borate buffer. They were stained with 2 μg ethidium bromide/ml solution and photographed under UV light.

3. RESULTS AND DISCUSSION

Digestion of nuclei or chromatin with trypsin gives a characteristic pattern of limit peptides for the histones [20,21]. Soluble chromatin was prepared from chicken erythrocytes and then digested with trypsin. The time-course of digestion was followed both by extraction with 5% perchloric acid (which extracts only histones H1 and H5 and their peptides) and by precipitation with trichloroacetic acid (which yields all chromosomal proteins). Fig.1B shows the rate of digestion of the lysine-rich histones set against a sample of pure histone H5 and a purified sample of the globular peptide of H5 obtained by a digestion in solution [9] and designated GH5: by 45 min the lysine-rich histones have been degraded to their limit peptides of which that from H5 presumably dominates. Detailed analysis of these limit products has shown that principally N-terminal residues are lost from core histones [21–24] and that the resulting core particle is not dramatically changed in structure [22].

From fig.1, digestion times of 10, 45 and 150 min were chosen for further digestion with micrococcal nuclease at 37°C to reduce it to core/chromatosome length. For each time of tryp-

sin digestion, 3 times of nuclease digestion were used: 10, 25 and 45 min, but only 6 of the combinations were analysed in sucrose gradients. During the nuclease digestion some precipitate formed and this was removed by centrifugation. The supernatants were loaded onto sucrose gradients and the protein and DNA content of monomers (~11 S) analysed on gels. In fig.2, the DNA lengths of the monomer fractions are displayed and it can be seen that at all times of digestion both the 167 bp band and the 144 bp band are present, a result typical of the micrococcal nuclease digestion of native chromatin [4,25]. It appears therefore that protection of the chromatosome length of DNA (167 bp) is almost as good in the monomer obtained from trypsin-digested chromatin as in native chromatin. In the same gel a control of an overnight nuclease digestion of trypsinised (45 min) chromatin is also shown. In this case the 144 bp band is much enhanced, showing that no inactivation of the nuclease occurred.

To be certain of the level of protein digestion in the monomer fractions the proteins were analysed following 20% trichloroacetic acid precipitation. In fig.3 the result of this analysis is shown on a nitrocellulose filter stained with amido black. In this way, an additional control is obtained on the transfer of proteins for subsequent analysis with anti-H5 antibody. Identical results appeared in gels stained with Coomassie blue (not shown). By comparison with fig.1 it can be seen that no further digestion with trypsin occurred during nuclease digestion and analysis in sucrose gradients, nor was any specific protein lost during the experiment.

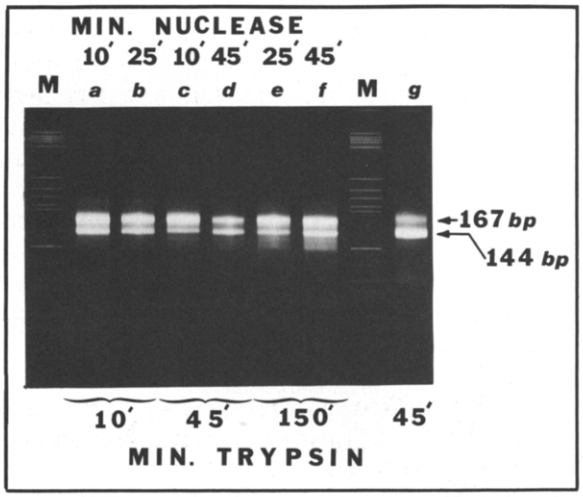

Fig.2. 10% polyacrylamide gels of duplex DNA extracted from monomer nucleosome fractions derived from 3 periods of trypsin digestion followed by 3 periods of micrococcal nuclease digestion, as shown (lanes a–f). Lane g is from a 45 min trypsin digestion of chromatin that was subsequently left overnight with micrococcal nuclease. The marker lanes are pBR 322/*Hae*III.

3.1. *Antibody identification of H5 peptides*

The isolated monomers that have a strong 167 bp band also show a strong protein component at the gel position of the limit peptide fragment of H5. Some uncertainty still remains however as to the identification of the H5 fragment in the monomer nucleosome since core histone products also run in this region of the gel [21]. Positive identification of the presence of the H5 fragment was obtained using an anti-H5 antibody. Fig.3 compares the proteins transferred to a nitrocellulose filter with the same filter incubated with anti-H5 antibody. It is thus possible to follow the level of H5 digestion before the nuclease digestion and in the monomers produced. From this approach we conclude that from the longest time of digestion (150 min) a chromatosome was purified with a similar level of protection of the 167 bp band as in chromatin, whilst the H5 histone was digested down to the level of its globular peptide GH5.

4. CONCLUSIONS

These data show clearly that if the lysine-rich histone H5 in chicken erythrocyte chromatin is cut back with trypsin to leave only the central globular fragment, subsequent digestion with micrococcal nuclease results in protection of 167 bp of DNA, as found in native chromatin. This implies that it is the central domain of H5 which is responsible for closing the second turn of DNA in the chromatosome as proposed [14] by the indirect approach of reconstitution.

Inspection of the protein gels reveals significant trypsin digestion of the core histones. Even at 45 min trypsin digestion and certainly at 150 min (for which very good protection of the 167 bp length remains) no intact H3 and very little intact H4 remains, although some H2A and H2B remain. It follows that the trypsin-susceptible domains

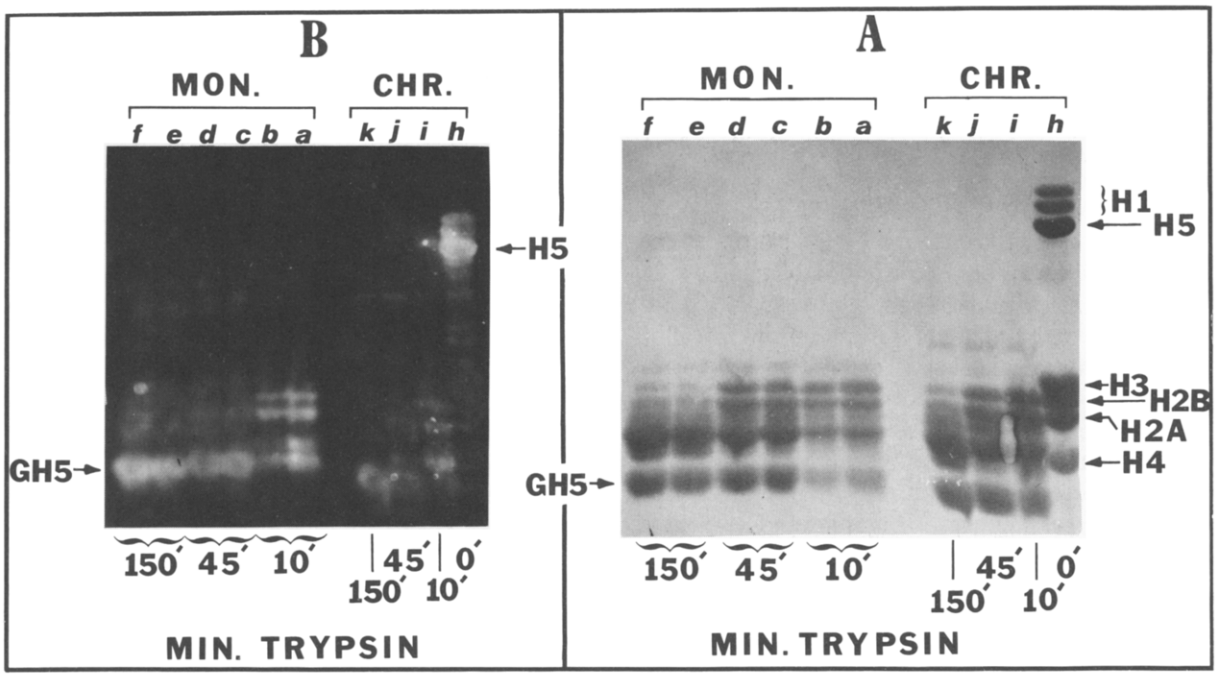

Fig.3. (B) The nitrocellulose filter of (A) blotted with rabbit anti-H5, developed with fluorescein-conjugated sheep anti-rabbit antibody and then washed to remove non-specific antibody binding. No binding to core histone is seen (lane h) and so all antibody binding to the lower region of the gel in monomers, lanes a–f, and in chromatin, lanes i–k, is due to the H5 fragment GH5 and possibly to a small extent to the analogous fragment from H1 (GH1). In the monomers from 10 min trypsin digestion there is evidence of defined intermediates in the degradation of H5 to GH5.

Fig.3. (A) 18% polyacrylamide gel of total proteins from nucleosomal monomers (lanes a–f) pre-treated with trypsin for the times shown and then with nuclease as in fig.2. The close similarity of lanes a–f to fig.1A shows that no further digestion of the histones occurred during nuclease digestion and centrifugation. Lanes h–k are total histone from the trypsin- and nuclease-treated (45 min) chromatin before centrifugation. Comparison with lanes a–f shows that no protein was lost in centrifugation. Lane h represents total histone control. Electrophoretic transfer to nitrocellulose stained with amido black.

(tails) of H3 and H4 which are mainly N-terminal [20,21] are not required to maintain the structure of the chromatosome, in the same way as it has been concluded that the trypsin-susceptible domains are not required for the maintenance of core particle structure [22,26].

ACKNOWLEDGEMENTS

P.P. acknowledges EMBO for the award of a short-term fellowship during the course of which part of this work was carried out. C.C.R. acknowledges the SERC of Great Britain.

REFERENCES

[1] Littau, V.C., Burdick, C.J., Allfrey, V.C. and Mirsky, A.E. (1965) Proc. Natl. Acad. Sci. USA 54, 1204–1212.
[2] Bradbury, E.M., Carpenter, B.G. and Rattle, H.W.E. (1973) Nature 241, 123–125.
[3] Billett, M.A. and Barry, J.M. (1974) Eur. J. Biochem. 49, 477–484.
[4] Noll, M. and Kornberg, R.D. (1977) J. Mol. Biol. 109, 393–404.
[5] Renz, M., Nehls, P. and Hozier, J. (1977) Proc. Natl. Acad. Sci. USA 74, 1879–1883.
[6] Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[7] Chapman, G.E., Hartman, P.G. and Bradbury, E.M. (1976) Eur. J. Biochem. 61, 69–75.

[8] Hartman, P.G., Chapman, G.E., Moss, T. and Bradbury, E.M. (1977) Eur. J. Biochem. 77, 45–51.
[9] Aviles, F.J., Chapman, G.E., Kneale, G.G., Crane-Robinson, C. and Bradbury, E.M. (1979) Eur. J. Biochem. 88, 363–371.
[10] Puigdomènech, P., Palau, J. and Crane-Robinson, C. (1980) Eur. J. Biochem. 104, 263–270.
[11] Barbero, J.L., Franco, L., Montero, F. and Moran, F. (1980) Biochemistry 19, 4080–4087.
[12] Giancotti, V., Russo, E., Cosimi, S., Cary, P.D. and Crane-Robinson, C. (1981) Biochem. J. 197, 655–660.
[13] Simpson, R.T. (1978) Biochemistry 17, 5524–5531.
[14] Allan, J., Hartman, P.G., Crane-Robinson, C. and Aviles, F.X. (1980) Nature 288, 675–679.
[15] Ruiz-Carrillo, A., Puigdomenech, P., Eder, G. and Lurz, R. (1980) Biochemistry 19, 2544–2554.
[16] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[17] Bowen, B., Steinberg, J., Laemmli, U.K. and Weintraub, H. (1980) Nucleic Acids Res. 8, 1–19.
[18] Perucho, M., Molgaard, H.V. and Ruiz-Carrillo, A. (1982) J. Biol. Chem. 257, 958–963.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] Weintraub, H. and Van Lente, F. (1974) Proc. Natl. Acad. Sci. USA 71, 4249–4253.
[21] Böhm, L., Briand, G., Sautière, P. and Crane-Robinson, C. (1981) Eur. J. Biochem. 119, 67–74.
[22] Whitlock, J.P. jr and Stein, A. (1978) J. Biol. Chem. 253, 3851–3861.
[23] Böhm, L., Crane-Robinson, C. and Sautière, P. (1980) Eur. J. Biochem. 106, 525–530.
[24] Böhm, L., Briand, G., Sautière, P. and Crane-Robinson, C. (1982) Eur. J. Biochem. 123, 299–303.
[25] Bakayev, V.V., Bakayeva, T.G. and Varshavsky, A.J. (1977) Cell 11, 619–629.
[26] Lilley, D.M.J. and Tatchell, K. (1977) Nucleic Acids Res. 4, 2039–2055.