Role of histones H1 and H3 in the maintenance of chromatin in a compact conformation

Study with an immobilized enzyme

Christian Marion, Bernard Roux and Pierre R. Coulet+

Laboratoire de Physico-chimie Biologique and +*Laboratoire de Biologie et de Technologie des Membranes du CNRS, Université Claude Bernard, Lyon I, 43, Bd. du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*

Received 28 April 1983

Chromatin polynucleosomes have been digested with trypsin immobilized on collagen membranes. This method allows the mild removal of the most accessible histone fragments simply by dipping the enzymatic membrane into the chromatin solution, without modification of its ionic and chemical composition. These results demonstrate that the removal of H1 does not affect the higher-order structure of chromatin and that only the elimination of the terminal regions of H3 leads to the unfolding of H1-depleted fibres. This observation suggests that structural changes reported in many previous works were not due to only the removal of H1 but to a concomitant unbinding from DNA of the N-terminal domain of H3.

Chromatin structure *Histone H1* *Histone H3* *Trypsin digestion* *Immobilized enzyme* *Enzymatic collagen membrane*

1. INTRODUCTION

Evidence has accumulated in the literature indicating that histone H1 is involved in the organization of chromatin fibres (reviews [1–3]). Some models have been proposed to account for this role and it has been suggested that H1 is localized at the point where DNA enters and exits from the core particle and that its presence is required to maintain the higher-order structure of chromatin [4]. Much of the evidence has come from electron microscopy and physico-chemical studies of chromatin after elimination of H1. In many cases the dissociation procedure was neither mild nor selective enough to prove that the structural changes observed were due only to the removal of H1 [5,6].

Our strategy has consisted in disrupting the chromatin structure by the use of proteases to assess the role that H1 plays in the condensation of chromatin [7]. Here, this approach has been improved by using trypsin immobilized on a collagen membrane [8,9]. With this method, the digestion is initiated simply by dipping the membrane into the chromatin solution at 25°C and the use of Ca^{2+} and of protease inhibitor is then avoided. This method allows the mild removal of the most accessible protein fragments without modification of their ionic environment, and provides valuable information about the role of each distinct part of histone molecules in the maintenance of chromatin superstructure. The results obtained by following changes in circular dichroism (CD) and electric birefringence properties indicate that the removal of H1 does not affect the structure of chromatin

Abbreviations: PMSF, phenylmethylsulfonyl fluoride; EDTA, ethylenediamine tetraacetic acid; TAME, *p*-toluene sulfonyl-L-arginine methyl ester; CD, circular dichroism

Published by Elsevier Science Publishers B.V.
00145793/83/$ 3.00

and that only the digestion of the terminal regions of H3 lead to the unfolding of the H1-depleted fibres.

2. MATERIALS AND METHODS

Rat liver nuclei were prepared as in [10]. Chromatin was briefly digested by micrococcal nuclease [11] and solubilized by lysis of the nuclei in 0.2 mM EDTA, 0.2 mM PMSF (pH 7.0). High-order oligomers were purified on a 5–28.2% isokinetic sucrose gradient in 1 mM sodium phosphate buffer, 1 mM EDTA (pH 7.4). After centrifugation for 18 h at 26000 rev./min in a SW 27 rotor, fractions containing oligomers with more than 10 nucleosomes were collected and dialyzed overnight against the standard buffer: 1 mM sodium phosphate, 0.2 mM EDTA (pH 7.4).

H1 was either dissociated from chromatin at high ionic strength and removed by fractionation on the gradient described above containing 500 mM NaCl or extracted with the ion-exchange resin AG 50 WX-2 [12] under different ionic conditions [5,12,13]:

(i) 1 mM sodium phosphate, 0.2 mM EDTA plus either 100 mM NaCl (buffer A) or 500 mM NaCl (buffer B);
(ii) 50 mM sodium phosphate, 0.2 mM EDTA plus either 50 mM NaCl (buffer C) or 100 mM NaCl (buffer D).

All H1-depleted samples were dialyzed extensively against the standard buffer.

Proteolytic digestion was performed by using trypsin covalently bound to the surface of a collagen membrane (2 cm × 2 cm), after an acyl-azide activation process [18]. This immobilization procedure which has wide potentialities [9] avoids the trypsin autolysis and the leakage of active protein which otherwise would contaminate the reaction medium. The immobilized enzyme activity determined by the rate of hydrolysis of TAME was equal to $0.2\,\mu mol.min^{-1}$ in the standard buffer (8 cm^2 area for both faces of the film).

The chromatin digestion was initiated by dipping the enzymatic membrane into 10 ml of the chromatin solution (A_{260} = 1.5) and stopped by taking the membrane out. The reaction medium was carefully thermostatted at 25°C and continuously stirred, in parallel with a control solution without membrane to check that no significant proteolytic degradation occurred.

SDS–16% polyacrylamide slab gels were run as described by Laemmli [14]. Circular dichroism measurements were obtained using a Dichrograph III–Jobin Yvon apparatus. The data are presented as molar ellipticity $[\theta]$ in $deg.cm^2.dmol^{-1}$. Procedures for transient electric birefringence experiments on chromatin solutions have been described [7,15,17].

3. RESULTS

3.1. *Dissociation of histone H1*

The protein analysis demonstrates that the removal of H1 is complete and that no measurable loss and degradation of the 4 core histone are observed (fig.1a). The CD spectrum of native chromatin showing correct DNA folding [18] changes drastically on the removal of H1 (fig.2a): the $[\theta]_{283}$ value is sharply increased, the negative peak around 295 nm disappears and the crossover point shifts from 268 to 262 nm.

It is now admitted that only native chromatin in a very compact conformation displays a positive birefringence in contrast to the negative ones of DNA and oligonucleosomes [7,19,20]. Then, the negative anisotropy reported by some authors can be explained by differences in the protein content or from a partial unfolding of nucleosomal chains. Typical birefringence oscillogram of H1-depleted chromatin is presented in fig.3. Upon the removal of H1 the sign of birefringence reversed becoming negative and more like that of DNA. Such a behaviour had been reported for purified oligonucleosomes [17].

3.2. *Digestion with immobilized trypsin*

The electrophoretic pattern of chromatin proteins in the course of trypsin immobilized treatment is shown in fig.1b. The order of digestion then observed accords with the fact that H1 is the most rapidly attacked and that H3 is the first histone of the core to be digested [11,21,22]. Concomitant with the disappearance of H3, the peptide P1 appears described as H3 residues 27–129 [23]. With increasing digestion time, the digest is very close to those published [21,23–27] and after 24 h incubation, all histone fractions were completely degraded.

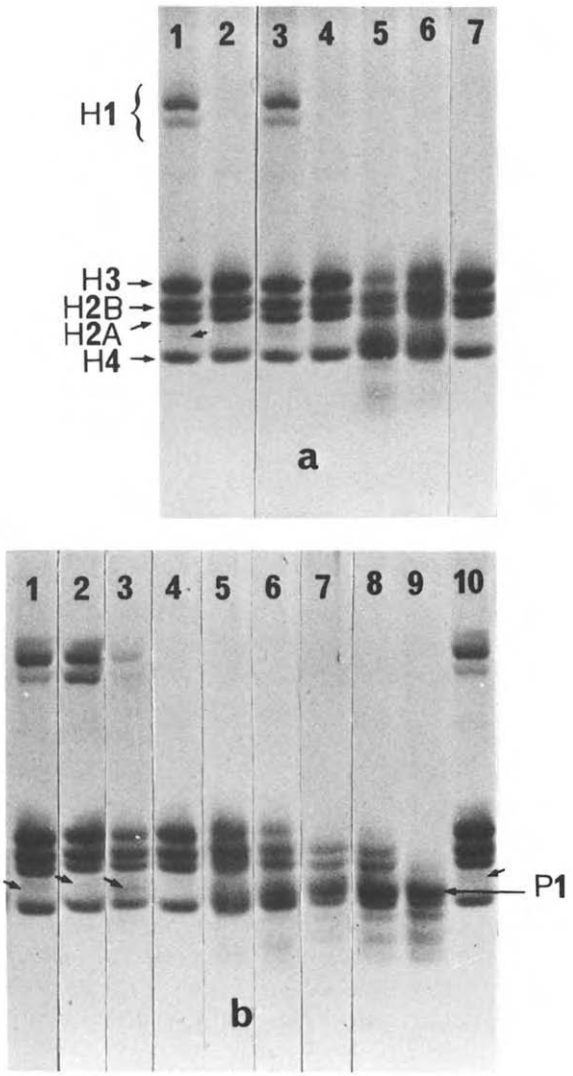

Fig.1. SDS–polyacrylamide gel electrophoresis of H1-depleted chromatin (a) and tryptic digests of chromatin (b). (a) Slots 1–7 refer, respectively, to native chromatin (1), chromatin-treated in high ionic strength (2) and treated with the resin in the presence of standard buffer (3), buffers A, B, C and D (4–7). (b) Slots 1–9: Chromatin digested with immobilized trypsin for 0, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 12 h. Slot 10 shows the analysis of chromatin incubated for 12 h without membrane. The arrow heads indicate the peptide P1′.

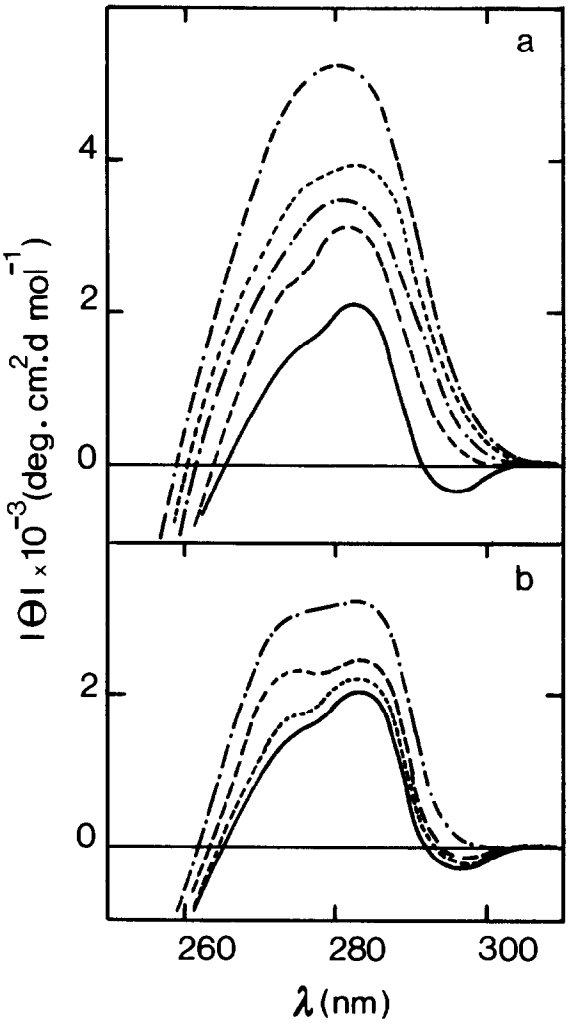

Fig.2. Circular dichroism spectra of H1-depleted (a) and trypsin digested chromatin (b). (a) Native chromatin (——) was treated with the resin in the presence of buffers A (– – –), B (– – – –), C (.—) and D (– – –), respectively. (– – –) refers also to the dissociation and removal of H1 on gradients in high ionic strength. (b) Native chromatin was digested with immobilized trypsin for 5 h (——), 7 h (– – –), 10 h (– – –) and 18 h (—.—).

We observed that the selective removal of H1 (after 4 h digestion) has no appreciable effect on the CD spectrum (fig.2b) and on the birefringence signal of native chromatin; in particular, the anisotropy remained positive (fig.3). With the extent of digestion and the appearance of P1, the CD spectrum shows a slight overall increase in ellipticity with a shift in the crossover point to 262 nm. In the meantime, a negative contribution to the birefringence appears and the signals become characteristic of the presence in the solution of components displaying opposite birefringence

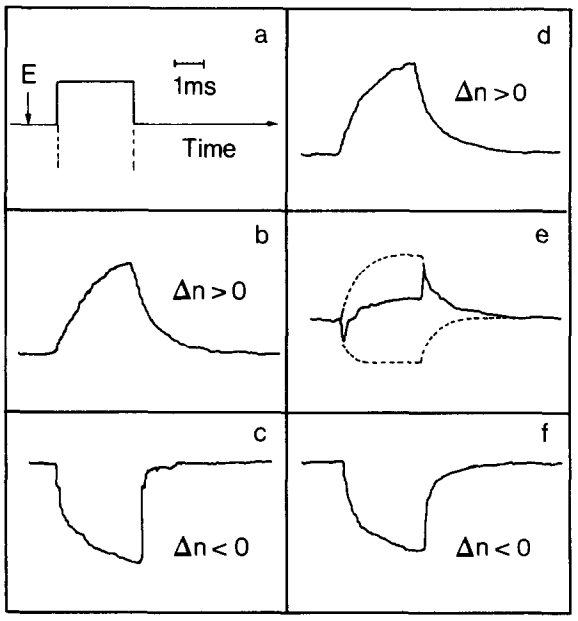

Fig.3. Typical oscillograms of the electric birefringence of chromatin. (a) Rectangular electric pulse; the field strengths applied varied from 0.5–3 $kV.cm^{-1}$; (b) native and (c) H1-depleted chromatin. (d), (e), (f) refer to chromatin digested with trypsin for 5 h, 7 h and 10 h, respectively. The amplitude of negative signals in (c) depends on the buffer used during the resin treatment and increases in the order A, C, D and B. The value with buffer A is identical to that obtained after a dissociation of H1 on gradients in high ionic strength.

signs [7]. After 8 h digestion all positive contribution has disappeared. The birefringence is entirely negative and its amplitude increased on digestion.

A marked change in the DNA conformation affecting the CD spectrum was only observed after 18 h incubation: $[\theta]_{283}$ was increased to 3400 $deg.cm^2.dmol^{-1}$ and the negative peak around 295 nm had disappeared.

4. DISCUSSION

In contrast to the removal of H1 by salt treatments, the mild and selective elimination of H1 with trypsin immobilized on a solid support does not lead to the disruption of the higher-order structure of chromatin. Structural change observed by the reversal of birefringence sign appeared only with the digestion of terminal regions of H3. This result demonstrated that these histone tails are easily accessible to proteases and directly involved in the maintenance of the 30 nm fibre structure. However, it is of particular interest to determine precisely which distinct domains of the H3 molecule plays the fundamental role in the organization and stabilization of nucleosomal DNA.

H3 is always the first histone of the core to be degraded [11,21,22] and its tryptic digestion results in two limit peptides: P1 representing residues 27–129 and P1′ the residues 21–135 containing the 6 C-terminal residues [23]. The finding that this C-terminal domain is easily accessible to proteases because in a very exposed location of the core particle [23,28,29] correlates well with the fact that P1′ is frequently observed in histone extracts from chromatin and very likely due to some autolytic digestion of chromatin [23].

Although our physical measurements on native chromatin and on samples incubated for a long time yield identical results while noting the presence of this faint band (fig.1), it is reasonable to conclude that the 6 C-terminal residues 130–135 of H3 are not involved in the stabilization of the superstructure.

Therefore it appears reasonable to propose that the N-terminal domain of H3 plays such a stabilizing role, as this had been suggested for the basic histone tails [2,30,33]. These NH_2-terminal regions which are not required for the folding of DNA within their own core particle [26,27,30] might interact stably in the 30 nm fibre with the DNA of the adjacent nucleosome [3,33]. The removal of these regions from core particle leads to reversible salt-dependent transitions due to a partial unfolding of this particle [27]. It has then been proposed that the amino-terminal domains, which have a cluster of basic residues resulting in a large net positive charge, cover nucleosomal DNA in a delocalized manner typical of counterions condensed by DNA [34]. This observation and the results presented here suggest that in many previous works on H1-depleted chromatin the effects reported were not due to the removal of H1 alone but to a concomitant unbinding from DNA of the N-terminal domain of H3.

Our results suggest that the interactions with DNA of this amino-terminal region of H3 may affect DNA supercoiling and then play an important role in the dynamic aspect of chromosome struc-

ture [35]; e.g., acetylation of histones has been correlated with gene transcription and it had been demonstrated that the major sites of this acetylation are in the basic NH_2-terminal tails which exhibit the greatest degree of sequence conservation [2]. The acetylation would neutralize positive charges of H3 and weaken the electrostatic interactions with DNA allowing the 30 nm fibre to adopt a more open conformation [3].

REFERENCES

[1] MacGhee, J.D. and Felsenfeld, G. (1980) Annu. Rev. Biochem. 49, 1115–1156.
[2] Sperling, R. and Wachtel, E.J. (1981) Adv. Protein Chem. 34, 1–60.
[3] Cartwright, I.L., Abmayr, S.M., Fleischmann, G., Lowenhaupt, K., Elgin, S.C.R., Keene, M.A. and Howard, G.C. (1982) CRC Crit. Rev. Biochem. 13, 1–86.
[4] Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[5] Modak, S.P., Lawrence, J.J. and Gorka, C. (1980) Mol. Biol. Rep. 6, 235–243.
[6] Thoma, F. and Koller, Th. (1981) J. Mol. Biol. 149, 709–733.
[7] Marion, C., Pallotta, L. and Roux, B. (1982) Biochem. Biophys. Res. Commun. 108, 1551–1558.
[8] Coulet, P.R., Julliard, J.H. and Gautheron, D.C. (1974) Biotechnol. Bioeng. 16, 1055–1068.
[9] Coulet, P.R. and Gautheron, D.C. (1981) J. Chromatogr. 215, 65–72.
[10] Hewish, D.R. and Burgoyne, L.A. (1973) Biochem. Biophys. Res. Commun. 52, 504–510.
[11] Noll, M., Thomas, J.O. and Kornberg, R.D. (1975) Science 187, 1203–1206.
[12] Bolund, L.A. and Johns, E.W. (1973) Eur. J. Biochem. 35, 546–553.
[13] Thoma, F. and Koller, Th. (1977) Cell 12, 101–107.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Marion, C. and Roux, B. (1978) Nucleic Acids Res. 5, 4431–4449.
[16] Roux, B., Marion, C. and Bernengo, J.C. (1979) in: Electro-optics and Dielectrics of Macromolecules and Colloids (Jennings, B.R. ed) pp.163–173, Plenum Press, New York.
[17] Marion, C. and Roux, B. (1980) Biochem. Biophys. Res. Commun. 94, 535–541.
[18] Lee, K.S., Mandelkern, M. and Crothers, D.M. (1981) Biochemistry 20, 1438–1445.
[19] Tjerneld, F., Norden, B. and Wallin, H. (1982) Biopolymers 21, 343–358.
[20] Houssier, C., Lasters, I., Muyldermans, S. and Wyns, L. (1981) Nucleic Acids Res. 9, 5763–5784.
[21] Weintraub, H. and Van Lente, F. (1974) Proc. Natl. Acad. Sci. USA 71, 4249–4253.
[22] Sollner-Webb, B., Camerini-Otero, R.D. and Felsenfeld, G. (1976) Cell 9, 179–193.
[23] Böhm, L., Briand, G., Sautière, P. and Crane-Robinson, C. (1981) Eur. J. Biochem. 119, 67–74.
[24] Tatchell, K. and Van Holde, K.E. (1977) Biochemistry 16, 5295–5303.
[25] Lilley, D.M.J. and Tatchell, K. (1977) Nucleic Acids Res. 4, 2039–2055.
[26] Whitlock, J.P. jr and Stein, A. (1978) J. Biol. Chem. 253, 3857–3861.
[27] Grigoryev, S.A. and Krasheninnikov, I.A. (1982) Eur. J. Biochem. 129, 119–125.
[28] Muller, S., Himmelspach, K. and Van Regenmortel, M.H.V. (1982) EMBO J. 1, 421–425.
[29] Klug, A., Rhodes, D., Smith, J., Finch, J.T. and Thomas, J.O. (1980) Nature 287, 509–516.
[30] Whitlock, J. and Simpson, R.T. (1977) J. Biol. Chem. 252, 6516–6520.
[31] Cary, P.D., Moss, T. and Bradbury, E.M. (1978) Eur. J. Biochem. 89, 475–482.
[32] Chahal, S.S., Mathews, H.R. and Bradbury, E.M. (1981) Nature 287, 76.
[33] McGhee, J.D., Rau, D.C., Charney, E. and Felsenfeld, G. (1980) Cell 22, 87–96.
[34] Manning, G.S. (1978) Quart. Rev. Biophys. 11, 179–246.
[35] Weisbrod, S. (1982) Nature 297, 289–295.