THE STRUCTURE OF PARTIALLY HISTONE DEPLETED NUCLEOHISTONE

C. SKIDMORE* and I.O. WALKER
Department of Biochemistry, Oxford University, Oxford, U.K.

and

J.F. PARDON and B.M. RICHARDS†
Searle Research Laboratories, High Wycombe, Bucks, U.K.

Received 1 March 1973

1. Introduction

Isolated DNA from eukaryotic nuclei shows no higher structural order than that of the double helix. When extracted as deoxyribose nucleohistone(DNH), X-ray diffraction shows that the DNA adopts a tertiary conformation which is thought to be a regular supercoil [1, 2]. Hydrodynamic studies show that a compact conformation also exists in solution [3]. Removal of the histones from the DNH results in the disappearance of the supercoil and a change to a more extended, flexible conformation in solution [3]; reconstruction by recombining DNA and histones regenerates the supercoil [4–6].

At least five types of histone are present in the native complex. The question can be posed as to whether the supercoil is dependent on the presence of all or only some of the histone types. This paper describes structural studies on whole DNH, F1-minus DNH, F1- and F3-minus DNH and DNH with only $F2a_1$ remaining attached. All samples were prepared by depletion of the native complex. The results show that the supercoil structure is not detectably changed by the removal of F1 or by subsequent removal of F3. Furthermore, when all histone types except $F2a_1$ are removed, supercoil molecules are still detectable. It is concluded that a single histone type, namely $F2a_1$, is capable of maintaining the supercoil without the interaction of other types of histone.

* Present address: The School of Pathology, The Middlesex Hospital Medical School, London W1P 7LD, U.K.
† To whom reprint requests should be sent.

2. Methods

Nucleohistone was prepared [7] and characterised as described previously [8]. Samples depleted of F1 and all fractions except $F2a_1$ were prepared by dialysing nucleohistone at room temp. against 0.7 M NaCl and 1.2 M NaCl, respectively, and then separating the dissociated protein by gel exclusion chromatography on Sepharose 4B [8]. DNH depleted of F1, F3 and F2B was prepared by incubating DNH with chymotrypsin (at a protein:enzyme ratio of 30:1) in 0.7 mM sodium phosphate (pH 7.0) for 3 hr at room temp. as described previously [9]. Bound peptide fragments were removed by dialysis against 0.7 M NaCl and chromatography on Sepharose 4B [9]. Protein:DNA ratios were measured using the Folin reaction or from the circular dichroic spectrum using the value of $\Delta\varepsilon$ at 220 nm, and the established linear relationship between $\Delta\varepsilon$ and protein concentration [10]. Protein was prepared from depleted samples as described previously [8, 9] and polyacrylamide gel electrophoresis (PAGE) was carried out using the method of Panyim and Chalkley [11]. Gels were stained with Coomassie Blue and destained by diffusion. Spectrophotometric scans were obtained with a Joyce–Loebl microdensitometer [9]. Scans were analysed with a Dupont curve analyser, as described previously [9]. Depleted samples were dialysed into 0.7 mM sodium phosphate (pH 7.0), then sedimented overnight at 48 K rpm in a Beckman/Spinco T50 rotor.

Fibre specimens for X-ray diffraction were drawn from the gelled pellet. Exposures were made using the

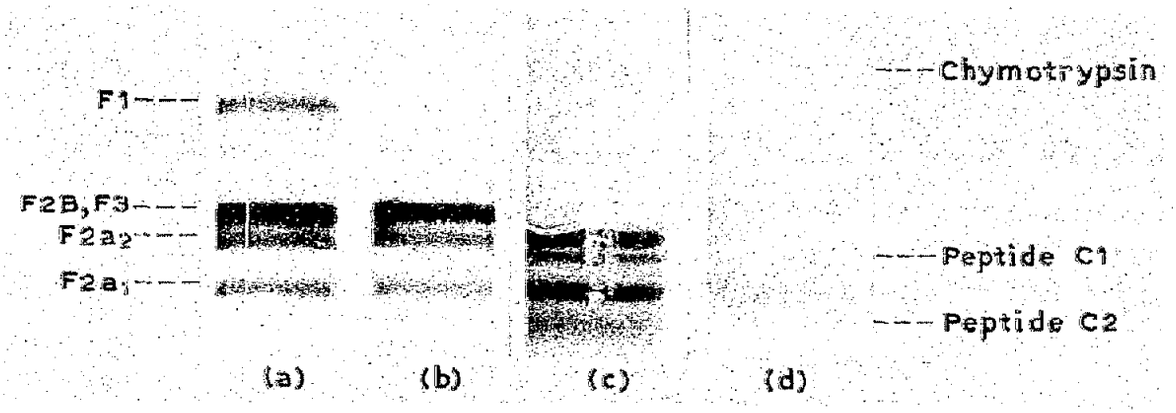

Fig. 1. Gel electrophoresis patterns of: a) Whole histone; b) histone extracted from F1 minus DNH; c) histone extracted from chymotrypsin-treated DNH; d) histone extracted from the fibre of the DNH depleted in 1.2 M NaCl which gave the diffraction pattern in fig. 2d. Between 10 and 40 mg of protein was loaded into the gels. Electrophoresis was for 3.5 hr on 10 × 0.6 cm gels at 2 mA/gel. Gels were stained in 0.1% Coomassie Blue in 7% acetic acid overnight and destained by diffusion against 7% acetic acid.

Searle X-ray camera (Baird and Tatlock, Ltd.) employing either Elliott toroidal mirror optics or Franks' double mirror optics. Exposure times were 20 hr at 98% relative humidity for the toroid optics using one-quarter of the toroid aperture. Similar times were used for the Franks' optics when studying the low angle diffraction pattern from dry specimens.

3. Results

3.1. F1-minus DNH

The PAGE pattern for this material is shown in fig. 1b. When compared with the pattern for whole histone (fig. 1a) the absence of histone F1 is clear. Quantitative analysis of the gel scans showed that less than 4% of other fractions had been removed. The diffraction pattern given by fibre specimens drawn from this material is shown in fig. 2b. It is indistinguishable from the pattern given by native DNH shown in fig. 2a. Both patterns show the series of "supercoil rings" corresponding to spacings of 22, 27, 37 and 55Å. The 110Å reflection which completes the series [5, 6] was not recorded by the camera technique used here.

3.2. Chymotrypsin-treated DNH

The gel pattern for this material (fig. 1c) shows that the chymotrypsin-treated sample lacks histones F1 and F3 and its content of histone $F2_b$ is greatly reduced. The remaining histones $F2a_1$ and $F2a_2$ are present in amounts not significantly different from those in whole histone. The peptides C1 and C2 are partial degradation products probably arising from F2b (see [9]) which remain bound to DNA in 0.7 M NaCl [9].

X-ray diffraction patterns given by this material, one of which is shown in fig. 2c, show significant orientation in the DNA component when compared with native DNA. Although not illustrated here, the same material when analysed in the dry state gives a diffraction pattern closely similar to that given by dry native nucleohistone.

3.3. DNA associated with histone $F2a_1$ alone

Dialysis against 1.2 M NaCl at room temp. and subsequent gel chromatography yields material from which all histones except $F2a_1$ are quantitatively removed. The histone:DNA ratio of this sample was 0.26. The PAGE pattern of the histone extracted from the drawn fibre which gave the X-ray diffraction pattern described below is shown in fig. 1d. Only one fraction, $F2a_1$, was present.

The X-ray diffraction pattern given by wet fibres of this material (fig. 2d) shows two sets of reflections. The first set is similar in all respects to that given by native DNH, while the second set is that expected from deproteinized DNA in the 'B' configuration. The pattern given by fibres of this material in the dry state (fig. 3) shows no detectable difference from that given by native DNH.

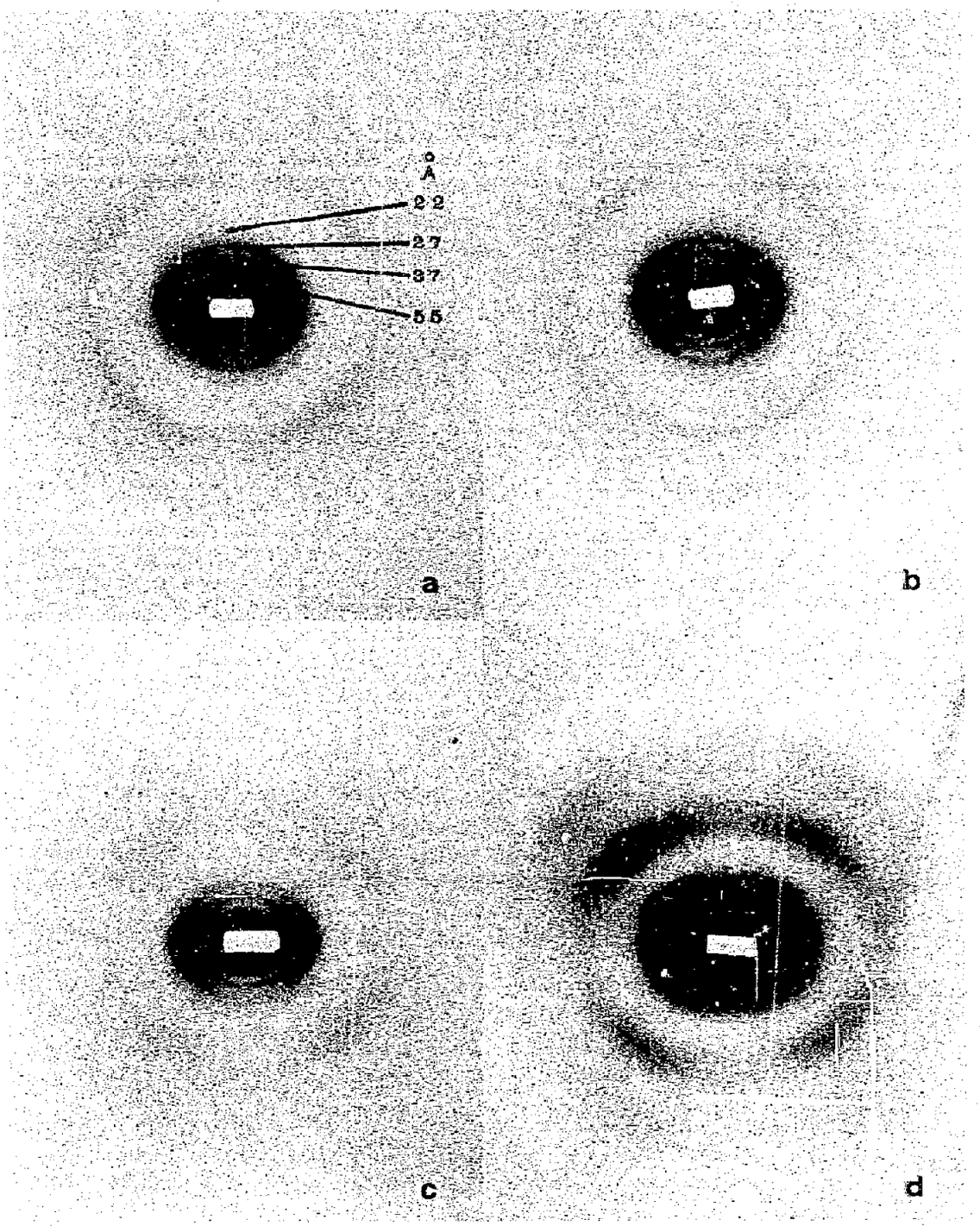

Fig. 2. Diffraction patterns obtained from fibre specimens of native DNH and partially histone depleted DNH. All patterns were obtained using toroid optics with a specimen-to-film distance of 7.5 cm.
a) Native DNH maintained at 95% r.h.;
b) F_1-minus DNH at 98% r.h.;
c) chymotrypsin treated DNH at 98% r.h.;
d) DNH lacking all histone types except $F2a_1$ at 100% r.h.

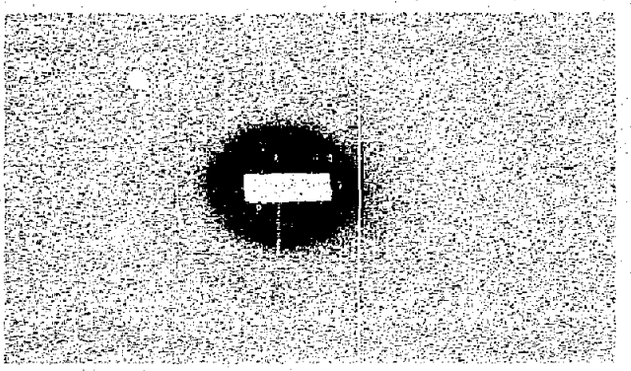

Fig. 3. Diffraction pattern obtained from a fibre of DNH lacking all histone types except $F2a_1$ at 0% r.h. The pattern was obtained using double mirror optics with a specimen-to-film distance of 10 cm.

4. Discussion

Removal of histone F1 from native DNH produces no detectable change in the structure of the protein–nucleic acid complex as studied by X-ray diffraction. This result agrees with that of Bradbury et al. [12] on F1-depleted DNH except that we find the low angle rings to be very well defined in patterns from both native and F1-depleted material. This observation shows that F1 is not required for the maintenance of the supercoil structure and supports the often quoted view that histone F1 attaches to DNA in a manner somehow differing from that of the remaining histones. Its inability to regenerate the supercoil when combined with DNA [5, 6] supports this possibility. It cannot be concluded on the basis of these observations that histone F1 plays no part in creating the supercoil in the cell [13].

Histones F1 and F3 comprise about 40% of the total protein content of DNH. When they are removed, together with the major portion of histone F2b following chymotrypsin treatment, a small but significant improvement in the orientation of reflections arising from deproteinized DNA is observed. This change in the X-ray diffraction pattern is very much more pronounced when only $F2a_1$ is left attached to the DNA. Patterns from this material show orientation arising from considerable amounts of deproteinized DNA as might be expected since about 80% of the histone protein has been removed. Far more important, however, is the observation that when only histone $F2a_1$ is left attached to the DNA, all other histone types having been removed, supercoil rings are clearly visible in the pattern from such material. This implies that the zones of DNA to which histone $F2a_1$ molecules attach remain in the supercoil conformation when all other histone types around them have been removed. It therefore appears that a single histone type can maintain the double helix in the tertiary supercoil conformation detected by X-ray diffraction.

A final comment is needed on the results of diffraction from dry samples. Densitometer traces show equivalent intensities for the 38 Å and 76 Å rings, respectively, in patterns from dry native and dry $F2a_1$-DNH. This result suggests that histone $F2a_1$ may be the only histone type responsible for giving rise to the dry pattern when it is attached to DNA.

The reason why several kinds of histone molecule are to be found in eukaryotic chromosomes remains obscure. The only known biological function of histones, namely that of causing the DNA to which they attach to adopt a tertiary conformation, seemingly can be fulfilled by one type of histone alone. The observations here described tell us only that this single type of histone can maintain the supercoil. Further work is needed to show whether one type of histone can, acting alone, generate the supercoil.

References

[1] J.F. Pardon, M.H.F. Wilkins and B.M. Richards, Nature 215 (1967) 508.
[2] J.F. Pardon and M.H.F. Wilkins, J. Mol. Biol. 68 (1972) 115.
[3] P. Hensen and I.O. Walker, European, J. Biochem. 22 (1971) 1.
[4] R.A. Garrett, J. Mol. Biol. 38 (1968) 249.
[5] B.M. Richards and J.F. Pardon, Expt. Cell. Res. 62 (1970) 184.
[6] J.F. Pardon and B.M. Richards, in: Chromosomes today, Vol. 3, eds. C.D. Darlington and K.R. Lewis (Longman Group Limited, London, 1972) p. 38.
[7] G. Zubay and P. Doty, J. Mol. Biol. 1 (1959) 1.
[8] P. Henson and I.O. Walker, European J. Biochem. 14 (1970) 354.
[9] S. Chattergee and I.O. Walker, European J. Biochem., in press.
[10] C. Skidmore, unpublished observations.
[11] S. Panyim and R. Chalkley, Arch. Biochem. Biophys. 130 (1969) 387.
[12] E.M. Bradbury, B.G. Carpenter and H.W.E. Rattle, Nature 241 (1973) 123.