

## THE EXISTENCE OF RANDOM COIL N-TERMINAL PEPTIDES – ‘TAILS’ – IN NATIVE HISTONE COMPLEXES

D. M. J. LILLEY\*, O. W. HOWARTH, V. M. CLARK

*Department of Molecular Sciences, University of Warwick, Coventry, CV4 7AL, UK*

and

J. F. PARDON and B. M. RICHARDS

*Searle Research Laboratories, Lane End Road, High Wycombe, HP12 4HL, UK*

Received 13 November 1975

### 1. Introduction

Histones interact with each other and with DNA [1–6]; both kinds of association are essential for the integrity of chromatin. Current models for the structure of chromatin are based upon a periodic repeating unit in which a segment of DNA comprising about 140–160 base pairs is folded with a packing ratio of 7 or less into a ‘bead’ containing eight histone molecules, two each of F2A1, F3, F2A2 and F2B [7,8]. Such chromatin ‘monomers’ are linked into a ‘thread of beads’ by the continuous DNA molecule [9,10]. DNase digestion initially cleaves the DNA of the linker region and permits the isolation of monomers and multiples thereof [11–15].

Complexes of histone molecules can be isolated either directly from chromatin [1,2] or from purified monomers. Using  $^{13}\text{C}$  nuclear magnetic resonance (n.m.r.) we have studied the conformation of individual histones when alone in solution [4,16] and in the grouping found in chromatin. In this paper we show that the histones in the group containing two molecules each of histones F2A1 and F3 have random coil N-terminal peptides which are ideally equipped to bind to DNA.

\* To whom communications should be addressed: Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, UK.

### 2. Materials

Chromatin gel was isolated from calf thymus nuclei which had been previously washed in saline-EDTA and disrupted under conditions of minimum shear [17]. Whole histone was extracted using 2 M NaCl and from it the tetramer containing histones F2A1 and F3 was isolated by fractionation on Sephadex G-100 and ammonium sulphate precipitation [18]. The purity was checked by means of polyacrylamide gel electrophoresis [19], chemical crosslinking [20] and sedimentation. Hen egg white lysozyme was obtained from Sigma (Grade I) and used without further purification.

### 3. N.m.r. spectra

$^{13}\text{C}$  n.m.r. proton decoupled spectra at 22.63 MHz were recorded on a Bruker WH-90 spectrometer. Samples were examined in 50 mM (histone) or 100 mM (lysozyme) NaCl solution containing 30%  $\text{D}_2\text{O}$  at 100 and 250 mg/ml respectively.

Using the theoretical analysis of Doddrell et al. [21], we have calculated that under our experimental conditions a  $^{13}\text{C}$  which is rigid with respect to the molecular frame should have a  $T_1$  of the order of 45 msec, whilst a random coil  $\text{C}_\alpha$  would be expected to have a much longer  $T_1$ , between 100–200 msec. Thus a pulse sequence [22]:

$180^\circ D_1$   $90^\circ D_2$  where  $D_1 \approx \ln 2 \times 45$  msec and  $D_2 > 0.5$  sec

will produce a null point in rigid frame  $^{13}\text{C}_\alpha$  resonances whereas mobile random coil  $^{13}\text{C}_\alpha$  resonances will possess inverted intensity. Such spectra will be referred to as partially relaxed Fourier transform (PRFT) n.m.r. spectra.

#### 4. Results and discussion

In order to use n.m.r. to prove or disprove the existence of histone 'tails' we have applied the following criteria:

(i) Extremely mild extraction techniques, avoiding extremes of pH and solvent composition, should be used in order to minimise histone complex denaturation;

(ii) Peptide backbone mobility should be examined.

Identification of side-chain mobility alone does not prove the existence of tails as, for example, mobile surface side-chains would be equally consistent with such data. For this reason we have turned our attention specifically to the mobility of  $\text{C}_\alpha$  carbons. Mobile  $\text{C}_\alpha$  carbons are consistent only with random coil peptides;

(iii) Relative mobility should be deduced from relaxation times. Previous n.m.r. investigations [4,16,23,24] of histones have relied upon differences in linewidth to estimate mobilities. This cannot be used as a rigorous test of backbone mobility since observed resonances from histones are generally composed of a number of partially overlapping resonances and hence  $\nu_{1/2}$  does not reflect  $T_2$  alone. This criticism applies particularly to the use of  $^1\text{H}$  linewidths in such studies, since even at high field strengths resolution is not good.

We have attempted to satisfy all these criteria by studying a native tetramer of histones F2A1 and F3 using a pulsed n.m.r. method which identifies mobile  $\text{C}_\alpha$  carbons by virtue of their comparatively long  $^{13}\text{C}$  spin-lattice ( $T_1$ ) relaxation times. We have compared the results obtained with similar ones from lysozyme which is known to be entirely globular in conformation.

As shown in fig.1 the  $^{13}\text{C}$  n.m.r. spectra for lysozyme and the histone tetramer are qualitatively quite

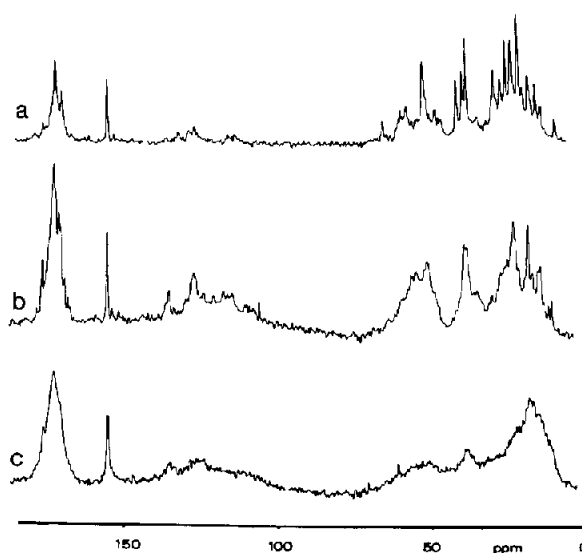


Fig.1.  $^{13}\text{C}$  n.m.r. spectra of; (a) histone F2A1-F3 tetramer at  $12^\circ\text{C}$ ; (b) lysozyme at  $42^\circ\text{C}$ ; (c) lysozyme at  $12^\circ\text{C}$ .

dissimilar. Spectra recorded from lysozyme at  $42^\circ\text{C}$  show only a few narrow resonances and that at  $12^\circ\text{C}$  only one, while many narrow resonances are evident in the histone spectrum. Clearly the overall structure of lysozyme is much less mobile than that of the histone tetramer even at elevated temperatures. The  $\text{C}_\alpha$  region of the spectra (45–60 ppm) from the two types of protein is markedly different, particularly at  $12^\circ\text{C}$ , that for lysozyme being considerably broader. Thus it is clear that the two species differ structurally in a fundamental way.

The nature of the structural differences between lysozyme and the F2A1-F3 histone tetramer is clarified when their PRFT spectra are compared, as shown in fig.2 and 3. In both cases the resonances of carbonyl and arginine  $\text{C}_\delta$  carbons remain inverted but this is merely a result of the absence of directly bonded protons. The aliphatic region (10–60 ppm) is of greater interest. It is seen that the PRFT spectrum of lysozyme contains fewer inverted resonances than in the corresponding histone tetramer spectrum, thereby indicating that the latter protein despite its higher molecular weight contains elements of considerable mobility. The most important region is that between 45–60 ppm assignable entirely to  $\text{C}_\alpha$  resonances. Lysozyme shows no inverted intensity in this

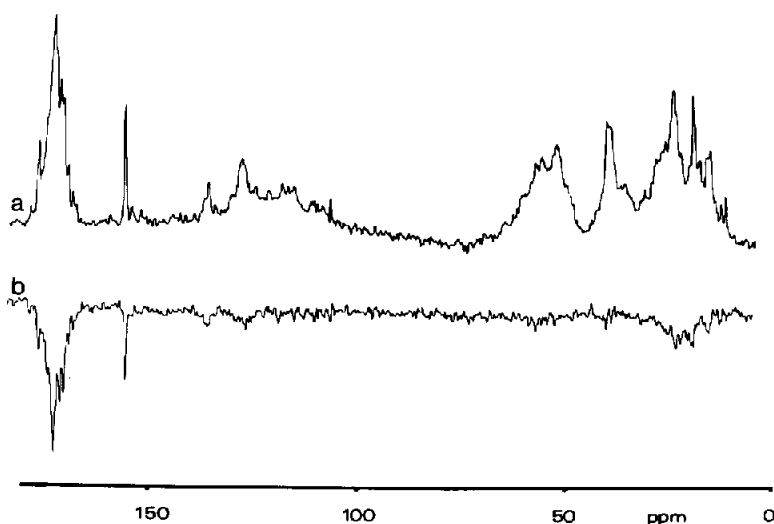


Fig.2. Normal (a) and PRFT (b)  $^{13}\text{C}$  n.m.r. spectra of lysozyme at  $42^\circ\text{C}$ .

region, which is consistent with its globular nature, while the F2A1-F3 tetramer does. This difference can be explained by postulating the presence of some  $\text{C}_\alpha$  carbons having mobilities much greater than the overall tumbling of the protein, which indicates that there are mobile random coil sections in the polypeptide backbone of the protein.

There are a number of reasons for suggesting this mobile region of the polypeptide backbone is restricted to the N-terminus. Firstly analysis of histone amino acid sequence by several methods [25,26] predicts that propensity to form secondary structure is lowest at the N-terminus. Secondly computer simulation of the  $^{13}\text{C}$  n.m.r. spectrum of the F2A1-F3

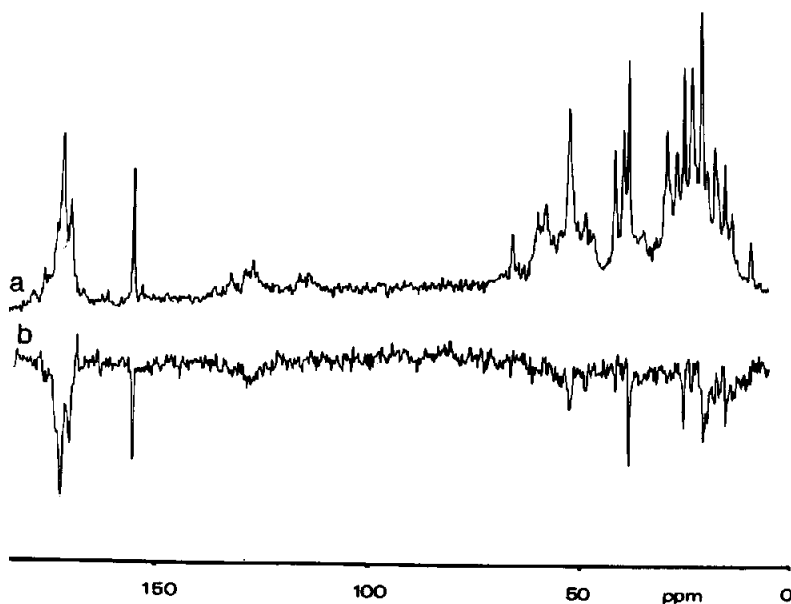


Fig.3. Normal (a) and PRFT (b)  $^{13}\text{C}$  n.m.r. spectra of histone F2A1-F3 tetramer at  $12^\circ\text{C}$ .

tetramer [27] indicates clearly that the N-terminal regions of the histones are the main contributors to the narrow part of the spectrum, i.e. that the mobility peculiar to the histones is localised to the N-termini. Thirdly the N-termini tend to be relatively more accessible to digestion by trypsin for histones in chromatin and after isolation [27,28].

Thus the existence of random coil 'tails' at the N-termini of the histones of the F2A1-F3 tetramer seems to be highly likely. An obvious role for such 'tails' is to provide sites for interaction with DNA via salt-type linkages between the positively charged basic residues of the protein and the negatively charged phosphates of the polynucleotide backbone. In this way groups of eight histones, possibly as two heterotypic tetramers (one molecule each of F2A1, F3, F2A2 and F3), are attached to each other predominantly via hydrophobic interaction and each histone molecule has a 'tail' for attachment to DNA. The resulting structure constitutes the bead unit in chromatin.

### Acknowledgement

We wish to thank Dr D. A. Couch for writing a program for our B-NC12 computer to perform PRFT experiments.

### References

- [1] Kelley, R. I. (1973) *Biochem. Biophys. Res. Commun.* 54, 1588-1594.
- [2] Kornberg, R. D. and Thomas, J. O. (1974) *Science* 184, 865-868.
- [3] D'Anna Jr., J. A. and Isenberg, I. (1974) *Biochemistry* 13, 4994-4997.
- [4] Clark, V. M., Lilley, D. M. J., Howarth, O. W., Richards, B. M. and Pardon, J. F. (1974) *Nuc. Acids Res.* 1, 865-880.
- [5] Bradbury, E. M. and Rattle, H. W. E. (1972) *Eur. J. Biochem.* 27, 270-281.
- [6] Adler, A. J., Ross, D. G., Chen, K., Stafford, P., Woiszwillo, M. J. and Fasman, G. D. (1974) *Biochemistry* 13, 616-622.
- [7] Kornberg, R. D. (1974) *Science* 184, 868-871.
- [8] Van Holde, K. E., Sahasrabudhe, C. G. and Shaw, B. R. (1974) *Nuc. Acids Res.* 1, 1579-1586.
- [9] Olins, A. L. and Olins, D. E. (1974) *Science* 183, 330-332.
- [10] Oudet, P., Gros-Bellard, M. and Chambon, P. (1975) *Cell* 4, 281-300.
- [11] Burgoyne, L. A., Hewish, D. R. and Mobbs, J. (1974) *Biochem. J.* 143, 67-72.
- [12] Rill, R. and Van Holde, K. E. (1973) *J. Biol. Chem.* 248, 1080-1083.
- [13] Noll, M. (1974) *Nature* 251, 249-251.
- [14] Finch, J. T., Noll, M. and Kornberg, R. D. (1975) *Proc. Nat. Acad. Sci. US.*
- [15] Shaw, B. R., Herman, T. M., Kovacic, R. T., Beaudreau, G. S. and Van Holde, K. E. (1975) *Proc. Nat. Acad. Sci. USA*, in the press.
- [16] Lilley, D. M. J., Howarth, O. W., Clark, V. M., Pardon, J. F. and Richards, B. M. (1975) *Biochemistry*, in the press.
- [17] Zubay, G. and Doty, P. (1959) *J. Mol. Biol.* 1, 1-20.
- [18] Van der Westhuyzen, D. R. and Von Holt, C. (1971) *FEBS Lett.* 14, 333-337.
- [19] Panyim, S. and Chalkley, R. (1969) *Arch. Biochem. Biophys.* 130, 337-346.
- [20] Davies, G. E. and Stark, G. R. (1970) *Proc. Nat. Acad. Sci. USA* 66, 651-656.
- [21] Doddrell, D., Glusko, V. and Allerhand, A. (1972) *J. Chem. Phys.* 56, 3683-3689.
- [22] Vold, R. L., Waugh, J. S., Klein, M. P. and Phelps, D. E. (1968) *J. Chem. Phys.* 48, 3831-3832.
- [23] Bradbury, E. M., Cary, P. D., Crane-Robinson, C. and Rattle, H. W. E. (1973) *Ann. N.Y. Acad. Sci.* 222, 266-289.
- [24] Bradbury, E. M., Cary, P. D., Crane-Robinson, C., Rattle, H. W. E., Boublik, M. and Sautière, P. (1975) *Biochemistry* 14, 1876-1885.
- [25] Pardon, J. F. and Richards, B. M. (1973) in: *Subunits in Biological Systems Part B*, pp. 1-70 and 305-322, (Fasman and Timasheff, eds.) Marcel Dekker, New York
- [26] Lewis, P. N. and Bradbury, E. M. (1974) *Biochim. Biophys. Acta* 336, 153.
- [27] Lilley, D. M. J., Howarth, O. W., Clark, V. M., Pardon, J. F. and Richards, B. M., in preparation.
- [28] Weintraub, H., Palter, K. and Van Lente, F. (1975) *Cell* 6, 85-110.