The structure and properties of histone F2a comprising the heterologous group F2a₁ and F2a₂ studied by ¹³C nuclear magnetic resonance

V.M. Clark*, D.M.J. Lilley, O.W. Howarth, B.M. Richards and J.F. Pardon'

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

Received 30 May, 1974

ABSTRACT

¹³C n.m.r. spectra have been obtained for aqueous solutions of histones F2a₁ and F2a₂, for the group F2a, for the appropriate amino acid mixtures and for the corresponding hydrolysates. These, when compared with computer simulated spectra give good agreement for secondary structure with that calculated from the known primary structure of the proteins. Evidence based on the spectra obtained at various salt concentrations leads to the conclusion that F2a is not a simple mixture but an interacting heterologous group of histones F2a₁ and F2a₂.

INTRODUCTION

Eukaryotic chromatin contains five types of histone in equal amounts (1). With the exception of histone F₁ the only known consequence of their attachment to DNA is the induction and maintenance of supercoiling of the double helix (2). To achieve this the different types may act conjointly and maybe co-operatively (3). Thus, there is increasing evidence that heterologous groups of histones are active in supercoiling, while individual histones are inactive in that they can combine with DNA but no supercoiling results (3,4).

Heterologous groups have been studied in terms of co-isolation (5), co-chromatographic behaviour and concentration-dependent sedimentation (6), salt-induced aggregation and configurational change (7). In all instances differences were found between the properties and behaviour of the group and those of the separated histone types which comprised the group. The simplest case is a group of two, although more complex groups

have been studied in some early work on reconstruction of nucleohistone studied by X-ray diffraction (3), and recently the requirement for more than one histone type in reconstruction has been emphasized (4).

In this paper we report the examination of the group of histones F2a₁ and F2a₂ by ¹³C nuclear magnetic resonance (n.m.r.) spectroscopy where we have tried to determine if the properties and behaviour of the heterologous group are distinguishable from those predicted by averaging the properties of the two component histone types.

EXPERIMENTAL

a) Preparation of Samples

Histones were extracted from calf thymus using the methods of Johns (8) and used within two weeks of preparation. F2a₂ was precipitated by the addition of two volumes of acetone, this giving an improved separation of F2a₁ from F2a₂. Before the final acetone wash, three ethanol washes were included to reduce the final content of guanidinium ion. Spectra were obtained immediately after the sample had been dried from the acetone used for storage. The purity of the histone fractions was checked by polyacrylamide gel electrophoresis.

b) Nuclear magnetic resonance spectroscopy

Proton noise-decoupled ¹³C n.m.r. spectra at natural abundance were recorded at 22.63 MHz on a Bruker WH-90 Fourier transform instrument. Histone samples were investigated as 100 mg/ml solutions in glass distilled water containing 30% deuterium oxide to provide a field lock. Ten millimetre spinning sample tubes were used, with probe temperatures of 280-290 K. Proton noise decoupling eliminated ¹³C - ¹H spin-spin splitting and provided a nuclear Overhauser enhancement. For each sample 1-3 x 10⁵ pulses were recorded.

c) Computer simulation

Spectra were simulated using published data (9-14) for ¹³C shifts in amino acids and proteins. In certain cases of

ambiguity, spectra of individual amino acids were recorded to aid the refinement of simulations. The program used contained the individual amino acid shifts and a parallel set of relative peak heights (scaled to allow for differential nuclear Overhauser enhancements) and received as its initial data set the amino acid composition of the protein. A Lorentzian line shape with an appropriate width was then assumed. The simulations were then refined by assuming that certain regions of the primary sequence are involved in secondary structure, the resulting constraints on internal motion in the protein producing greater linewidths for resonances of carbon atoms contained within these regions (15,16). Thus, these resonances were simulated using a line-width twenty times that of the 'free' carbon atoms, and a height reduced by this factor and also by a further factor to account for the reduction in the nuclear Overhauser enhancements (15,17). The regions of primary sequence which were considered to be immobilized in the random coil or helical conformation were varied, the more closely to approach agreement between simulated and observed spectra.

d) Hydrolysis of histone samples

Samples (120 mg) of histones F2a₁ and F2a₂ were subjected to complete hydrolysis in 6 M hydrochloric acid at 100°C for 24 hours in sealed evacuated tubes. The hydrolysed samples were concentrated by placing in an evacuated desiccator at 100°C for two hours. The resulting concentrates were each dissolved in 1 ml glass distilled water containing 30% deuterium oxide, and the pH adjusted to 6.9 by the addition of 2 M sodium hydroxide solution.

RESULTS AND DISCUSSION

The proton-decoupled ¹³C n.m.r. spectra of histones F2a₁ and F2a₂ are shown in figures 1a and 1b respectively. About twenty peaks are clearly resolvable in each spectrum, and, with the exception of the extreme low field (carbonyl) region, these

Figure 1: Natural abundance proton-decoupled ^{13}C n.m.r. spectrum of (a) histone F2a₁ in H_2O (1.4×10^5 accumulations) and (b) histone F2a₂ in $\text{H}_2\text{O}/30\% \text{D}_2\text{O}$ (2.89×10^5 accumulations). The chemical shift scale (for these and all subsequent spectra) is relative to external TMS. The peak marked by † is due to the methyl resonance from deuterioacetone which was used to provide a lock for spectrum (a) only.

have widths of 10-20 Hz. This is rather broader than normally observed for a single carbon resonance and may be attributable in part to several resonances having closely similar, but not identical, chemical shifts contributing to the lineshape. The spectra may be divided into the following approximate regions:

- (i) upfield from 23 ppm, containing most of the resonances from the side chain methyl groups,
- (ii) the region from 23 to 50 ppm, including the resonances from aliphatic methylenic carbons,
- (iii) the region from 50 to 70 ppm, containing the peptide backbone C_{α} resonances,
- (iv) the region from 110 to 140 ppm, containing those of aromatic carbons,
- (v) the single, comparatively sharp resonance at 157 ppm attributable to the guanidinium carbon of the arginine side chain,
- (vi) the broad envelope from 165 to 180 ppm, containing all the backbone carbonyl carbon resonances.

The ^{13}C spectra of completely hydrolysed samples of histones F2a₁ and F2a₂ are shown in figures 2a and 2b respectively. Whilst the signal-to-noise ratios are lower than those of the spectra of the native proteins, many more peaks are observable and linewidths are considerably narrower, being less than 10 Hz. The spectrum of hydrolysed F2a₂ is similar to that shown in figure 3 for a mixture of amino acids having the same molar ratio.

The simulated spectra of the amino acid mixtures (figure 4) were found to be closely comparable to those of the hydrolysed proteins. They are, however, substantially different from those of the spectra of the native histones. Hence refinement was carried

Figure 2: Natural abundance proton decoupled ^{13}C n.m.r. spectra of a total hydrolysate of (a) F2a₁, pH 6.9, in $\text{H}_2\text{O}/20\% \text{D}_2\text{O}$ (1.3×10^5 accumulations) and (b) F2a₂, pH 6.9, in $\text{H}_2\text{O}/20\% \text{D}_2\text{O}$ (1.4×10^5 accumulations).

Figure 3: Natural abundance proton-decoupled ^{13}C n.m.r. spectrum of a mixture of amino acids in the same polar proportions as occurring in F2a₂, in $\text{H}_2\text{O}/30\% \text{D}_2\text{O}$ (2.7×10^5 accumulations).

Figure 4: Simulated proton-decoupled ^{13}C n.m.r. spectra for (a) F2a₁ and (b) F2a₂, assuming equal contributions from each part of the primary sequence.

Figure 5: Simulated proton-decoupled ^{13}C n.m.r. spectra of (a) F2a₁ assuming that residues 1-26 are freely mobile in solution, whilst residues 27-102 are involved in secondary structure, with a resulting reduction in mobility, and (b) for F2a₂, assuming that residues 1-39, 71-82 and 117-129 are mobile in solution, whilst residues 40-70 and 83-116 are involved in secondary structure.

out by assuming that specific segments of the molecules were involved in secondary structure. The best fit obtained for the two histones are shown in figures 5a and 5b, and it is evident that close agreement has been achieved. The fit for F2a₁ is estimated to be accurate to two residues for the positioning of the helix-coil boundary. For example, moving the boundary towards the N terminal end results in marked changes in the simulation due to loss of high field isoleucine resonances. The best simulation for F2a₁ obtained so far is that in which the polypeptide chain is free from the N-terminus to residue 26 and coiled from residue 27 to the C-terminus. The best simulation of the F2a₂ spectrum was produced assuming regions of free polypeptide between residues 1-39, 71-82 and 117-129 inclusive, and regions of secondary structure between residues 40-70, and 83-116 inclusive. The helix-coil boundary closest to the N-terminus is sensitive to changes in exact positioning due to the resonance of tyrosine 39 which is required for a good simulation of the aromatic region, but the remaining boundaries are rather less closely defined and cannot be estimated with an accuracy closer than five residues. Bearing in mind that a complete examination of all possible helix-coil divisions is impracticable, it may be noted that the regions of secondary structure proposed for these histones are in very good agreement with those predicted (18) using the methods of Prothero (19) and Schiffer and Edmundson (20) and hence confidence may be expressed in the structures presented here.

The ¹³C proton-decoupled natural abundance spectrum of histone F2a is shown in figure 6. This shows similarities to the spectrum of F2a₂ and to that obtained by summing the best simulations for its component histones F2a₁ and F2a₂ shown in figure 7. Comparison of this with the spectra recorded for three different F2a samples indicates that, within the experimental limits imposed by the unfavourable signal-to-noise ratios, the spectrum of F2a may be adequately described as a sum of its component parts.

Figure 6: Natural abundance proton-decoupled ^{13}C n.m.r. spectrum of F2a in $\text{H}_2\text{O}/30\% \text{D}_2\text{O}$ (3.1×10^5 accumulations).

If any specific F2a₁-F2a₂ interactions occur in solution it is probable that these involve those portions of the molecules which give rise to broad signals in the pure samples, i.e. the α -helical regions. The spectrum of a sample containing equal amounts of histones F2a₁ and F2a₂ shown in figure 8 is not significantly different from that of F2a.

Bradbury and his colleagues (21) have shown that the signal-to-noise ratio of ¹H n.m.r. spectra of histone F2a₁ varies with concentration of added sodium chloride. We have observed comparable variations in the ¹³C n.m.r. spectra of both F2a₁ and F2a₂. The signal-to-noise ratios (normalised with respect to zero salt concentration) are plotted against sodium chloride molarity in figure 9 for these proteins and for F2a. The effect of increasing salt concentration is most marked for F2a₁, the spectrum of which is almost indistinguishable from noise at 0.2 M NaCl, whilst the F2a₂ and F2a spectra are still distinguishable at 0.4 M NaCl. The spectra of histones in various NaCl concentrations do not, however, appear to show significant qualitative differences from the zero salt spectra. This suggests that, at the high protein concentrations used, no differences in secondary structure occur on addition of salt and thus the resulting aggregation does not selectively involve any particular regions of the protein. Figure 9 also suggests that F2a, rather than behaving as a sum of its component histones, appears to be more resistant than are its individual components to aggregation effects caused by the increasing concentration of salt. This suggests that, at the protein concentrations used, F2a is not a simple mixture of F2a₁ and F2a₂ but must contain some complex of the two component histones. If F2a were just a simple mixture of F2a₁ and F2a₂, then at 0.2 M NaCl its spectrum would be indistinguishable from that of F2a₂ at 0.2 M NaCl. These spectra shown in figures 10a and 10b respectively are clearly not identical. This confirms our contention that F2a is an interacting heterologous pair of histones.

Figure 7: Simulated proton-decoupled ^{13}C n.m.r. spectrum produced by addition of the best simulations obtained for F2a₁ [fig. 5(a)] and F2a₂ [fig. 5(b)].

Figure 8: Natural abundance proton-decoupled ^{13}C n.m.r. spectrum of a $\text{H}_2\text{O}/30\% \text{D}_2\text{O}$ solution $5\text{m mol} \cdot \text{l}^{-1}$ in both F2a₁ and F2a₂ (2.5×10^5 accumulations).

Figure 9: Plot of signal-to-noise ratio against sodium chloride concentration (all solutions 10m mol l⁻¹ protein in H₂O/30% D₂O). Signal-to-noise ratios have been corrected to be equivalent to 10⁵ accumulations and then normalised with respect to that at zero salt concentration. Each point presented is an average of measurements on at least two different samples, 1, F2a₁; 2, F2a₂; 3, F2a; 4, average of 1 and 2.

Figure 10: Natural abundance proton-decoupled ^{13}C n.m.r. spectra of (a) F2a in 0.2 mol l^{-1} sodium chloride solution in $\text{H}_2\text{O}/30\% \text{ D}_2\text{O}$ (2.5×10^5 accumulations) and (b) F2a₂ in 0.2 mol l^{-1} sodium chloride solution in $\text{H}_2\text{O}/30\% \text{ D}_2\text{O}$ (2.4×10^5 accumulations).

ACKNOWLEDGEMENTS

We thank Twyford Laboratories for a Fellowship (D.M.J.L.)

REFERENCES

1. Johns, E.W. (1967) Biochem.J. 104, 78-82
2. Pardon, J.F. and Wilkins, M.H.F. (1972) J.Mol.Biol. 68, 115-124
3. Richards, B.M. and Pardon, J.F. (1970) Expt.Cell.Res. 62, 184-196
4. Kornberg, R.D. and Thomas, J.O. Private Communication
5. Pardon, J.F. and Richards, B.M. (1972) in Chromosomes Today, Vol. 3, Ed. Darlington and Lewis, pp.38-46, Longman, London
6. Kelley, R.I. (1973) Biochem.Biophys.Res.Comm. 54, 1588-1594
7. D'Anna, J.A. and Isenberg, I. (1973) Biochemistry 12, 1035-1043
8. Johns, E.W. (1967) Biochem.J. 105, 611-614
9. Dwek, R.A. (1973) Nuclear Magnetic Resonance in Biochemistry, pp.154-156, Oxford University Press, London
10. Horsley, W., Sternlicht, H., and Cohen, J.S. (1970) J.Amer.Chem.Soc. 92, 680-686
11. Bradbury, J.H. and Norton, R.S. (1973) Biochim.Biophys.Acta., 328, 10-19
12. Freedman, M.H., Lyster, J.R., Chaiken, I.M. and Cohen, J.S. (1973) Eur. J.Biochem. 32, 215-226
13. Glushko, V., Lawson, P.J., and Gurd, F.R.N. (1972) J.Biol.Chem. 247, 3176-3185
14. Freedman, M.H., Cohen, J.S. and Chaiken, I.M. (1971) Biochem.Biophys.Res.Comm. 42, 1148-1155
15. Doddrell, D., Glushko, V. and Allerhand, A. (1972) J.Chem.Phys. 56, 3683-3689
16. Browne, D.T., Kenyon, G.L., Packer, E.L., Sternlicht, H. and Wilson, D.M. (1973) J.Amer.Chem.Soc. 95, 1316-1323
17. Allerhand, A. and Oldfield, E. (1973) Biochem. 12, 3428-3433
18. Pardon, J.F. and Richards, B.M. (1973) in Subunits in Biological Systems Part B, pp.1-70 and 305-322, Ed. Fasman and Timasheff, Marcel Dekker, New York
19. Prothero, J.W. (1966) Biophys.J. 6, 367-370
20. Schiffer, M. and Edmundson, A.B. (1967) Biophys.J. 7, 121-135
21. Boublik, M., Bradbury, E.M. and Crane-Robinson, C. (1970) Eur.J.Biochem. 14, 486-497

Searle Research Laboratories, Lane End Road, High Wycombe, Bucks.

* to whom reprint requests should be sent