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The Structure of Sea-Urchin-Sperm Histone ϕ1 (H1) in Chromatin and in Free Solution

Trypsin Digestion and Spectroscopic Studies

Pere PUIGDOMENECH, Jaume PALAU, and Colyn CRANE-ROBINSON

Instituto de Biologia Fundamental, Universidad Autonoma de Barcelona, and Biophysics Laboratories, Portsmouth Polytechnic

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The lysine-rich H1-type histone ϕ1 from the sperm of the sea urchin *Arbacia lixula* has been subjected to tryptic digestion in free solution at high ionic strength. Tw trypsin-resistant peptides Gϕ1 and LGϕ1 have been isolated, comprising 81 and 93 amino acids respectively, i.e. about a third of the intact histone. Circular dichroism and 270-MHz proton NMR have been used to demonstrate that the smaller peptide Gϕ1 contains all the secondary and tertiary structure of intact histone ϕ1. It is concluded that the resistant peptides represent a compact folded portion of the histone ϕ1 chain, whilst the remaining two-thirds is disordered in free solution. Trypsin digestion of *Arbacia lixula* nuclei, under conditions where histone ϕ1 remains tightly bound to the chromatin, leads to the same resistant peptide Gϕ1. From this it is concluded that in chromatin the chain region corresponding to Gϕ1 is likewise folded and inaccessible to trypsin, whilst the remaining exposed residues are located periferally and probably in an extended conformation. The rather unusual H1-type histone ϕ1 from sea urchin sperm thus has a three-domain structure like calf thymus H1 and chicken erythrocyte H5. The present data emphasise the fact that this three-domain structure exists in chromatin and is not merely a free-solution artefact.

Considerable progress in the understanding of chromatin structure has been made in recent years through the use of nucleases to probe DNA conformation. The use of proteases to study the conformation of chromosomal proteins has received less attention. Experiments on the trypsin digestion of the core histones in chromatin [1, 2] have led to the conclusion that the N-terminal regions of these histones are preferentially digested. Although the structures underlying the high exposure of these parts of the core histones are not known, these results are in accord with the concept of a structural distinction between the N-terminal and C-terminal regions of the histones that has followed from studies of the $(H3/H4)_2$ tetramer and $(H2A/H2B)_2$ dimer in free solution [3–5]. In that work it was shown that the N-terminal regions of the histone complexes are not compacted, but free and mobile in solution. A similar approach has been taken in the study of the conformation of histone H1 in free solution. Tryptic digestion was shown to result in preferential digestion of 35 N-terminal residues and all the C-terminal half of the molecule beyond residue 120 [6]. The remaining central portion of the molecule was shown to contain all the secondary and tertiary structure of intact H1 and moreover to retain this structure after removal of the N-terminal and C-terminal regions. A three-domain structure containing a single central folded region has also been demonstrated for histone H5 in a similar manner [7], although in this case the N-terminal region was 21 residues long, rather than 35 residues.

Free solution studies of histone conformation are subject to the uncertainty that since the histone has been removed from the other components with which it interacts in chromatin, the results may not reflect the native conformation. In the case of the lysine-rich histones H1 and H5 it is likely that they interact only with DNA and not with the other histones and therefore can reasonably be studied in solution in the absence of other histones. To study the effect of DNA binding on the conformation of lysine-rich histones we have digested H5 · DNA reconstituted complexes with trypsin and observed the same protection of the central region (residues 21–100) as for free H5 [8]. Although this result strongly suggests that the N-terminal and C-terminal regions of H5 are extended and exposed and remain conformationally distinct regions when H5 binds to DNA, the situation is not

necessarily identical to that in chromatin. For this reason we have carried out trypsin digestion studies of nuclei, as well as of free solution H1, and compared the results. The tissue used was sea urchin sperm. This material is of particular interest since the nucleosomal repeat of 241 base-pairs [9] is the longest reported and a current hypothesis has it that the length of the linker DNA depends on the nature of the H1 present.

The H1 histone from sea urchin sperm [10] (often termed ϕ1) contains a very high proportion of arginine (11%) and in this respect resembles H5. The sequence is partially known [11] and despite considerable differences from other 'very-lysine-rich' histones, sequence homology between these proteins and calf thymus H1 is clear and well defined [12].

EXPERIMENTAL PROCEDURE

Histone ϕ1 was prepared from sea urchin *Arbacia lixula* sperm heads by 5% perchloric acid extraction as previously described [10, 13]. The protein was stored as a dry acetone precipitate; when used for spectroscopic studies it was dissolved initially in water (pH $\approx$ 4) and then appropriate volumes of salt solutions were added to reach the desired ionic strength and finally the pH was adjusted. Nuclei were prepared from washed fresh sperm by homogenization in a Potter-Elvehjem homogenizer with 0.3 M sucrose, 10 mM Tris, 0.1 mM phenylmethylsulphonyl fluoride, 5 mM $MgCl_2$, 0.5% Triton X-100, pH 7.4.

Trypsin digestion of histone ϕ1 in solution was carried out for 2.5 h at 24 °C in 1 M NaCl, 10 mM Tris, pH 7.4 or 8.0. Trypsin (treated with *N*-tosyl-L-phenylalanyl chloromethyl ketone), at 40 units/mg, was dissolved in buffer at 1 mg/ml and a volume was added to the protein solution (at 5 mg/ml) to achieve a trypsin-to-protein ratio of 1/1000 by weight. Digestion was followed as a function of time by measuring the turbidity at 400 nm of aliquots dissolved in 20% trichloroacetic acid solution. For preparative purposes the digestion was stopped by the addition of an excess of *N*-tosyl-L-lysyl chloromethylketone and by lowering the pH from 8.0 to 3.0 with concentrated HCl. Separation of peptides was carried out on a Sephadex G-50 column. Peptides were precipitated with 8 vol. acetone after dialysis against 10 mM HCl. The precipitates were then washed with acetone and vacuum-dried at room temperature.

Trypsin digestion of Triton-X-100-washed nuclei was carried out on a nuclear suspension in 70 mM phosphate, pH 8 (I = 0.19 M) for 90 min at 4 °C. The ratio of trypsin to histone ϕ1 was 1:250 (w/w). This ratio was determined by measuring the absorbance at 260 nm of an aliquot of nuclei lysed with 1% sodium dodecylsulphate and assuming a 1:1 ratio of total histone to DNA. The concentration of histone ϕ1 in the nuclear suspension was approximately 0.2 mg/ml.

Electrophoresis with sodium dodecylsulphate was carried out in 15% polyacrylamide slab gels following the method of Laemmli [14].

Amino acid analyses were carried out using hydrolysis in 6 M HCl at 110 °C for 24 h.

Proton Nuclear Magnetic Resonance

Spectra were recorded at 270 MHz on a Bruker WH-270 Fourier transform spectrometer. Samples (10 mg/ml) were dissolved directly in ^2H_2O and then appropriate amounts of concentrated salt solutions were added. The pH was changed by adding volumes of concentrated NaO^2H or ^2HCl. pH measurements were made in the NMR tube with a Pye-Ingold microelectrode.

Circular dichroic spectra were recorded on a Cary 61 spectropolarimeter in 1-cm path-length cells with samples at a concentration of 0.1 mg/ml.

RESULTS AND DISCUSSION

Trypsin Digestion of Histone ϕ1 in Free Solution

Histone ϕ1 prepared from *Arbacia lixula* sperm as described above was digested with trypsin at pH 8.0, 24 °C, at an ionic strength of 1 M and a protein concentration of 5 mg/ml. It has previously been demonstrated that at high ionic strength ($\approx$ 1 M NaCl), histone ϕ1 folds to form secondary and tertiary structure [13]. The progress of digestion was followed by turbidity and by gel electrophoresis (Fig. 1). The plot of turbidity (as apparent absorbance at 400 nm) shows two zones: up to about 40 min digestion is rapid, but thereafter is considerably slower. The electropherogram in Fig. 1 shows that by about 30 min most of the histone ϕ1 has been degraded down to two peptides LGϕ1 and Gϕ1. Further digestion of these products is much slower, in particular Gϕ1 is very resistant to trypsin action. Since at short digestion times (5 and 10 min) a very large number of digestion products are formed, it follows that a large section of the histone ϕ1 chain is very susceptible to trypsin digestion and degradation is not a result of high susceptibility at one or two 'special' basic residues. When the limiting digestion products LGϕ1 and Gϕ1 are slowly further degraded by trypsin no such series of peptides is observed. It is concluded that part of the histone ϕ1 chain has a disordered and trypsin-accessible conformation, whilst the remainder, representing peptides LGϕ1 and Gϕ1 is tightly folded and relatively inaccessible to trypsin.

The two peptides LGϕ1 and Gϕ1 were separated for preparative purposes using a Sephadex G-50 column at high ionic strength essentially as described

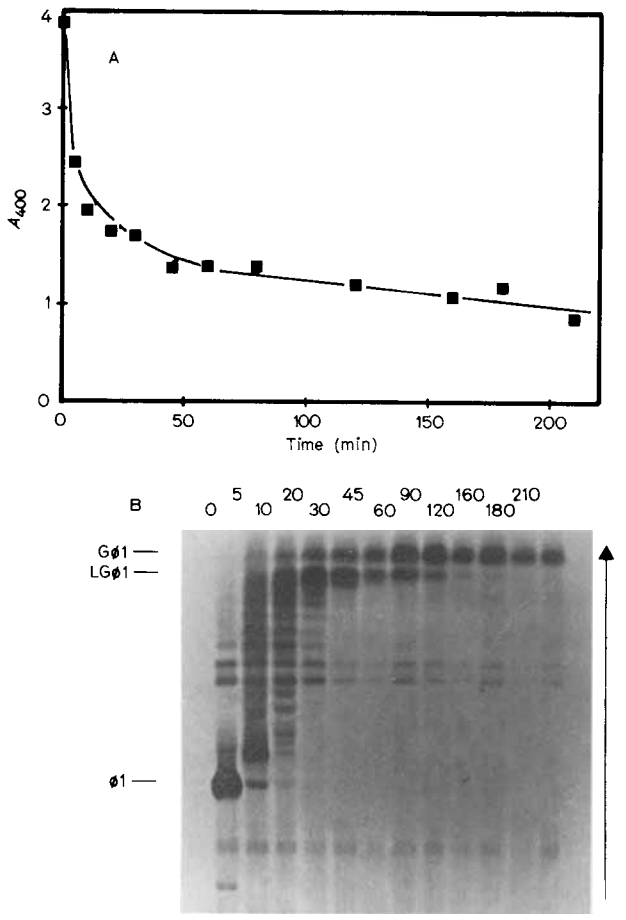

Fig. 1. *Kinetics of digestion of* Arbacia lixula *sperm histone ϕ1 in 1 M NaCl, 10 mM Tris, pH 8, 25 °C.* (A) Turbidity (as apparent absorbance at 400 nm) of aliquots in 20% trichloroacetic acid at 4 °C plotted against time. (B) Dodecylsulphate gel electrophoresis of total protein from the above experiment

previously for peptides from histone H5 [7]. Fig. 2 shows that the relative proportions of LGϕ1 and Gϕ1 can be altered by changing the pH of the trypsin digestion. At pH 7.4 approximately equal amounts of both peptides result and separation by Sephadex G-50 is effective. Pure Gϕ1 can also be prepared by increasing the tryptic activity using pH 8.0.

The molecular weight of the two peptides LGϕ1 and Gϕ1 has been assessed from their mobilities in dodecylsulphate gel electrophoresis relative to several standards. The standards used were ovalbumin, β-lactoglobulin, histones H3, H2B, H2A and H4, uteroglobin. A plot of log M_r against mobility (Fig. 3) yields molecular weights for Gϕ1 and LGϕ1 of 9400 and 10600 $\pm$ 600. Table 1 gives amino acid compositions of ϕ1, LGϕ1 and Gϕ1 as molar percentages. The compositions of the two peptides have been converted to numbers of residues by normalisation to a value of two tyrosine residues. That intact histone ϕ1 contains two tyrosines, one phenylalanine and two histidines was demonstrated previously from the NMR spectrum of the aromatic protons [13] and spectra of both Gϕ1 and LGϕ1 (see below) are identical to intact ϕ1 in this respect. Following normalisation of the amino acid compositions to two tyrosines, the values for histidine and phenylalanine correspond satisfactorily to two and one residue respectively in both peptides. The values for methionine are not satisfactory in that the smaller peptide appears to have one more residue than the larger peptide. This is probably due to too low a value in the larger peptide resulting from methionine oxidation. The equivalent protein from the sperm of *Parechinus angulosus* is known to contain four methionine residues [15]. Normalisation leads to molecular weight estimates of 8600 for Gϕ1 and 9800 for LGϕ1. These are in satisfactory agreement with the values obtained from electrophoresis. The peptide LGϕ1 is larger by about 12 residues (Ala_5,Lys_3,Asp_1,Thr_1,Ser_1,Arg_1).

The Conformation of the Peptide Gϕ1

Earlier studies [6, 7] of the structure of the peptides excised from histones H1 and H5 by trypsin digestion showed that they contained all the secondary and tertiary structure found in the intact parent molecule. The same finding is reported here for sea urchin sperm histone ϕ1. Tertiary structure is monitored by high-resolution NMR and secondary structure by circular dichroism. Fig. 4 demonstrates that the ring-current shifted methyl resonances (numbered 1, 2, and 3) that are observed in intact ϕ1 (at pH 3, 1 M NaCl) (spectrum C) are also observed in peptides Gϕ1 and LGϕ1 under the same conditions. These peaks are characteristic of the tertiary structure and are completely absent from disordered Gϕ1 at pH 3 with no added salt. At pH 7.3 shifted methyls 1 and 2 are displaced somewhat, but the spectrum remains essentially unchanged. A considerable number of other resonances also show characteristic changes in chemical shift on folding (e.g. threonine methyls at about 1.2 ppm) that are clearly the same for the peptides as for the parent ϕ1 molecule. The aromatic protons are shown in Fig. 5. At pH 3, 1 M NaCl, the two histidine C2H protons of Gϕ1 and LGϕ1 are at their characteristic shifts of 8.7 and 8.8 ppm as found in intact ϕ1 [13]. Likewise the C-3,5 protons of the two tyrosine residues are found at characteristic shifts of 6.7 and 6.8 ppm [13]. From both the upfield and the aromatic spectrum it is concluded that the tertiary structure of intact histone ϕ1 is essentially preserved in both peptides Gϕ1 and LGϕ1.

Peptide NH Exchange Rates

Another way in which NMR can be used to assess the presence of secondary and tertiary structure in a protein is the observation of peptide NH protons

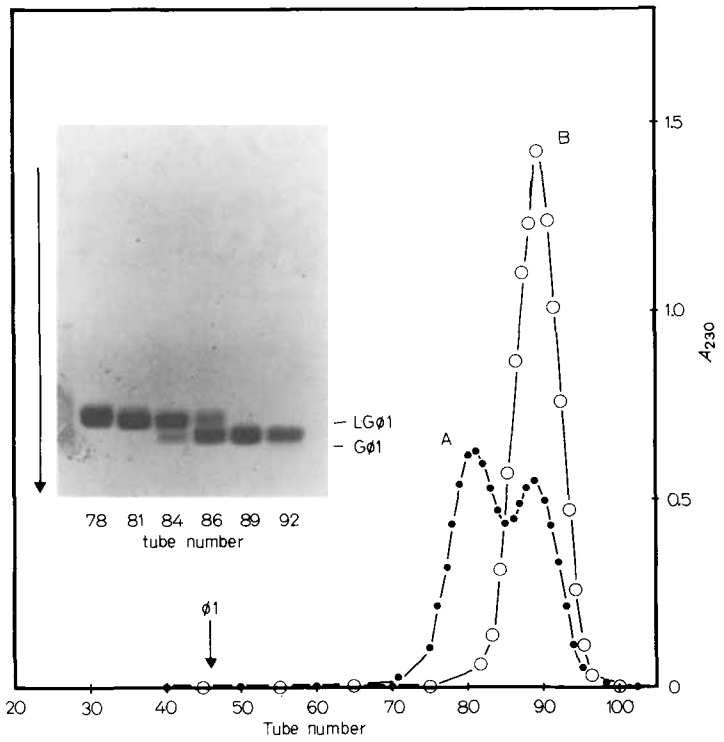

Fig. 2. *Gel exclusion chromatography of tryptic digests of histone ϕ1 in free solution.* The column (150 × 3 cm) of Sephadex G-50 was equilibrated with 0.5 M NaCl, 10 mM HCl. 6-ml fractions were collected at a flow rate of 50 ml/h. Curve A: digestion at pH 7.4. Curve B: digestion at pH 8. The inset shows dodecylsulphate gel electrophoresis of fractions from curve A. Also shown is the elution volumn of intact histone ϕ1

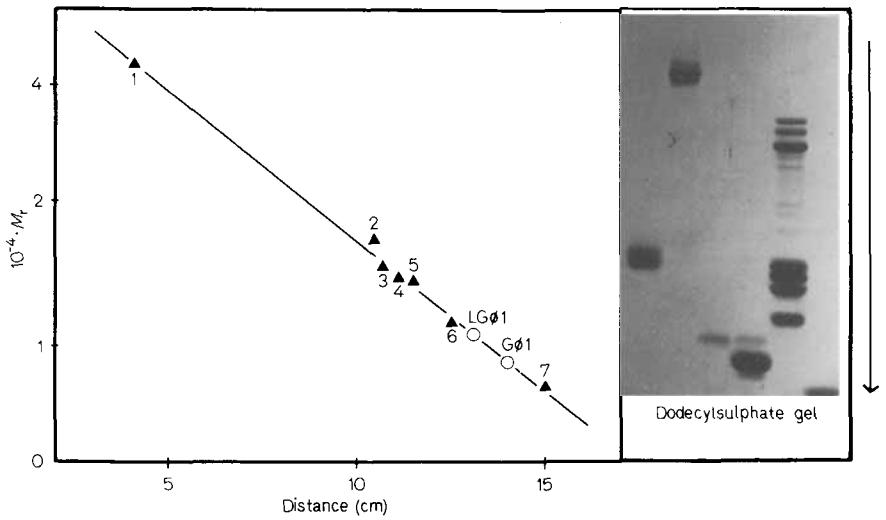

Fig. 3. *Calibration dodecylsulphate electrophoresis used to estimate to the molecular weight of peptides LGϕ1 and Gϕ1.* The standards used were: (1) ovalbumin M_r 45000; (2) β-lactoglobulin M_r 17500; (3) histone H3 M_r 15300; (4) histone H2B M_r 14000; (5) histone H2A M_r 13800; (6) histone H4 M_r 11300; (7) uteroglobulin (reduced) M_r 8000. The position of the standard proteins 1–7 on the gel corresponds to their position on the vertical molecular weight axis of the graph. The molecular weights calculated for Gϕ1 and LGϕ1 were 9400 and 10600 ± 600, the correlation coefficient of the plot being 0.993

that exchange slowly due to structural shielding. By dissolving histone ϕ1 and the peptide Gϕ1 directly in 1 M NaCl solution at pH 3, considerable peptide NH resonance is observed and the rate at which this disappears due to exchange with ^2H_2O of the solvent can be followed. Fig. 6 shows a logarithmic plot of the decay of two signals (at 7.63 and 8.48 ppm). The spectrum in Fig. 6 of histone ϕ1 in 1 M NaCl, pH 3, was obtained 1 h after dissolution whilst that shown in Fig. 5(C) was the spectrum after 2 h. By comparing the area of the resonance at 8.48 ppm in Fig. 5 with that of single histidine C-2 protons

Table 1. *Amino acid composition of peptides LGϕ1 and Gϕ1 compared with ϕ1 histone*
The composition of *ϕ1* is taken from our previous work [13]. The number of residues have been calculated in all cases supposing two tyrosines. The nearest integer is given in parentheses

Amino acid	ϕ1	LGϕ1	LGϕ1	Gϕ1	Gϕ1
	mol/100 mol	mol/100 mol	Number of residues	mol/100 mol	Number of residues
Asp	2.2	5.2	4.7 (5)	5.0	4.1 (4)
Thr	2.5	6.4	5.8 (6)	5.8	4.7 (5)
Ser	6.7	6.0	5.4 (5)	5.4	4.4 (4)
Glu	2.0	3.6	3.3 (3)	4.1	3.3 (3)
Pro	9.0	5.8	5.3 (5)	5.9	4.8 (5)
Gly	4.6	10.6	9.6 (10)	12.1	9.8 (10)
Ala	23.6	17.1	15.6 (16)	13.5	10.9 (11)
Val	2.8	6.3	5.8 (6)	7.1	5.7 (6)
Met	1.2	3.6	3.3 (3)	5.2	4.2 (4)
Ile	2.8	7.3	6.6 (7)	8.2	6.7 (7)
Leu	1.4	2.9	2.6 (3)	3.6	2.9 (3)
Tyr	0.8	2.2	2.0 (2)	2.5	2.0 (2)
Phe	0.4	1.1	1.0 (1)	1.4	1.1 (1)
Lys	27.4	13.8	12.5 (12–13)	11.8	9.5 (9–10)
His	1.2	2.1	1.9 (2)	2.3	1.9 (2)
Arg	11.2	6.1	5.6 (6)	6.1	4.9 (5)
Total			(92–93)		(81–82)

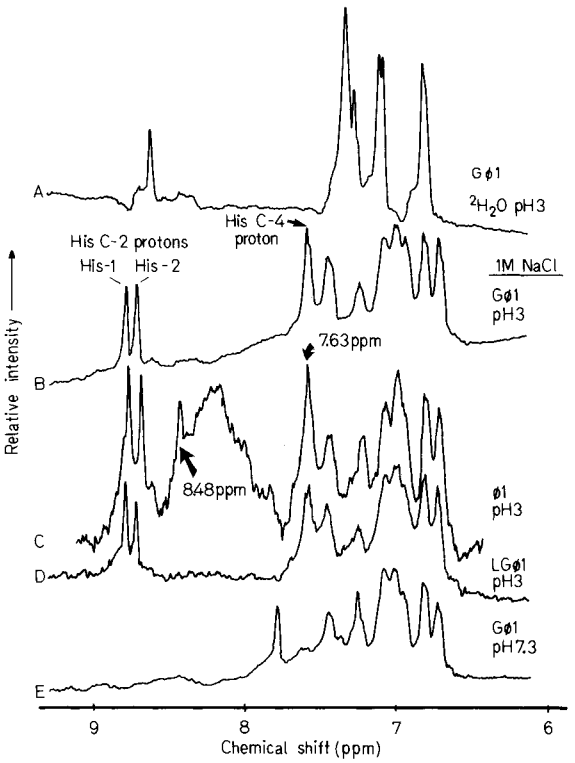

Fig. 5. *270-MHz lowfield NMR spectra of histone ϕ1 and its peptides.* (A) Peptide Gϕ1 disordered at pH 3.0; (B) peptide Gϕ1 folded at pH 3.0, 1 M NaCl; (C) intact ϕ1 folded at pH 3.0, 1 M NaCl; (D) peptide LGϕ1 folded at pH 3.0 1 M NaCl; (E) Peptide Gϕ1 folded at pH 7.3, 1 M NaCl. The broad resonance between 7.8 and 8.6 ppm corresponds to more rapidly exchanging peptide NH protons and the two peaks at 7.63 and 8.48 ppm correspond to 'hard-to-exchange' NH protons. Spectrum C obtained after 2 h in ^2H_2O, 1 M NaCl

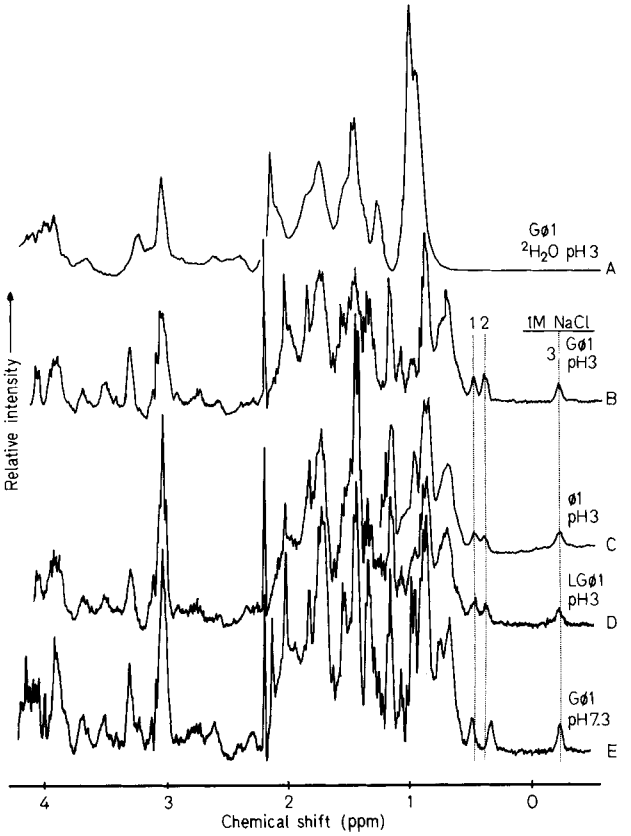

Fig. 4. *270-MHz upfield NMR spectra of histone ϕ1 and its peptides.* (A) Peptide Gϕ1 disordered at pH 3.0; (B) peptide Gϕ1 folded at pH 3.0, 1 M NaCl; (C) intact ϕ1 folded at pH 3.0, 1 M NaCl; (D) peptide LGϕ1 folded at pH 3.0, 1 M NaCl; (E) peptide Gϕ1 folded at pH 7.3, 1 M NaCl. Vertical lines indicate ring-current-shifted methyls 1, 2, and 3

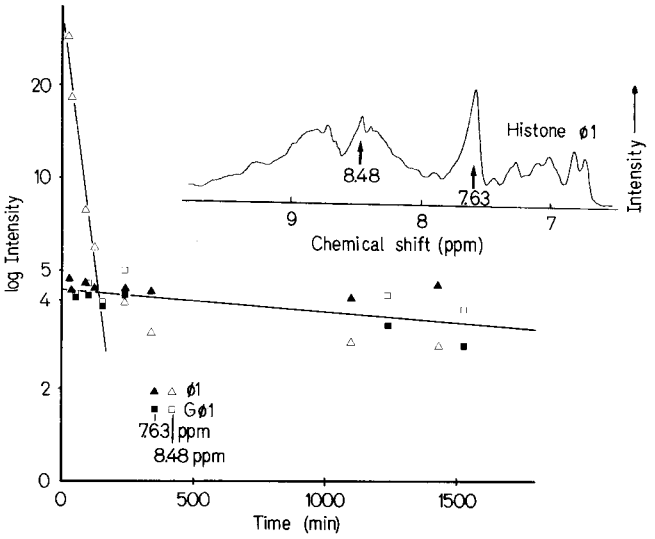

Fig. 6. *Log plot of peak intensity of two 'hard-to-exchange' NH protons in intact ϕ1 (triangles) and in Gϕ1 (squares).* Inset shows the spectrum of intact ϕ1 after 1 h in ^2H_2O, 1 M NaCl. The two slopes correspond to $\tau_{1/2}$ values of 45 min and 280 h

it follows that this peak represents a single peptide NH proton on top of a broad envelope of other NH protons. The decay of intensity at 8.48 ppm consists of two components having half lives of about 45 min and about 280 h in intact ϕ1. In the peptide Gϕ1 only the slowly exchanging component is observed, having a similar half-life to that found in intact ϕ1. The rapidly exchanging background ($\tau_{1/2} \approx 45$ min) is attributed to regions of histone ϕ1 that are removed by the trypsin. The sharp resonance is attributed to a shielded peptide NH. The observation that this shielded NH is present in both intact ϕ1 and the peptide Gϕ1 and shows a similar slow exchange rate in both is taken to mean identity of structure. The situation is akin to the comparison of shifted methyl resonances in intact ϕ1 and the peptide Gϕ1 as indicators of tertiary structure, except that for the peptide NH, two parameters can be compared, the chemical shift and the exchange rate. The slowly exchanging peak at 7.63 ppm (see Fig. 5 and 6) also shows a similar exchange rate in both intact ϕ1 and the peptide Gϕ1. In this case the resonance lies on top of the two histidine C-4 proton peaks at pH 3, (see Fig. 5, spectrum B) so that after a long time interval the intensity does not fall to zero. It is not certain that this exchangeable proton is a peptide NH, it might be a side-chain NH or OH proton, but its appearance in both ϕ1 and Gϕ1 with a similar and slow exchange rate and with the same chemical shift is a strong indication of identity of structure.

Secondary Structure

Circular dichroism was used to compare the number of helical residues in peptide Gϕ1 with intact histone ϕ1. The concentration of the protein samples was obtained from the absorption at 280 nm since it is known from the NMR spectrum that both ϕ1 and Gϕ1 contain two tyrosine residues ($\varepsilon_{Tyr} = 1350\,cm^{-1}\,mol\,Tyr^{-1}$) and no tryptophan. Helicity was obtained from the ellipticity at 222 nm using a value of -1000 deg. cm^2 dmol^{-1} as characteristic of the random coil [16] and -30000 deg. cm^2 dmol^{-1} as characteristic of 100% helix [17]. It has previously been shown that at high ionic strength, ϕ1 does not contain β structures [13]. The measured ellipticity of intact ϕ1 at 222 nm was -4700 deg. cm^2 dmol^{-1} and this represents 12.7% helicity, or 28 residues if the protein is taken as consisting of a total of 223 residues [15]. The measured ellipticity of Gϕ1 was -11500 deg. cm^2 dmol^{-1} at 222 nm and this represents 36.2% helicity or 30 residues on the assumption that Gϕ1 contains a total of 82 residues (see Table 1). It is concluded that trypsin digestion has not removed any of the secondary structure.

Histidine Environments

In globular proteins the imidazole protons of the several histidine residues typically exhibit different chemical shifts and pK_a values as a result of their being in different structural environments. These two parameters of the histidine residues are therefore characteristic of the tertiary structure. The C-2 protons of the two histidine residues of histone ϕ1 (which are both present in the peptide Gϕ1) do not have an identical chemical shift at pH 3 (see Fig. 5) and on titration exhibit different pK_a values. Fig. 7 shows a plot of histidine C-2 proton chemical shifts against pH for both ϕ1 and Gϕ1. Peak His-1 exhibits a pK_a of 5.6 in both ϕ1 and Gϕ1, whilst peak His-2 exhibits a pK_a of 6.3 in ϕ1 and 6.6 in Gϕ1. Thus the two histidine residues maintain their particular chemical shift and pK_a values in the peptide Gϕ1. (The difference of 0.3 in the pK_a of His-2 between ϕ1 and Gϕ1 is within the present experimental error.) This argues for identity of structure in the peptide Gϕ1 and intact ϕ1.

From Fig. 4 it can be seen that there is a pH-dependent change in the chemical shift of ring-current shifted methyls 1 and 2. This is plotted in the upper part of Fig. 7. In the case of methyl 2 the changes in chemical shift clearly take place between pH 6 and 7 and therefore correlate with His-2. Since there is no overall large-scale change in the structure over the pH range 3–8 (no change in the ellipticity is observed and very few proton resonances change chemical shift), the effects on methyl 2 must be structurally very localised. This implies that the residue giving rise to methyl 2 lies close in the tertiary fold to the histidine of peak His-2. In the case of methyl 1 the change of chemical shift with pH is very much

less than for methyl 2 and there is not an exact accord between peptide and parent protein. It is not possible therefore to decide whether methyl 1 is close to the histidine residue of peaks His-1 or His-2.

No change of tyrosine chemical shift was observed in the pH range 3–8 (unlike histone H5 [18]).

Tryptic Digestion of Nuclei

Sea urchin sperm nuclei were digested with trypsin at an enzyme-to-substrate ratio of 1:250. This is four times higher than for the experiments on isolated histone ϕ1 in free solution and was chosen to give comparable digestion times, in the range 1–2 h, for the two experiments. The ionic strength of the medium (0.19 M) is that for which chromatin is maximally condensed [19] and is well below that at which dissociation of histone ϕ1 takes place (I = 0.6 M). The products of digestion were treated with 5% perchloric acid (which extracts histone ϕ1 preferentially from normal, undigested nuclei), or with 0.25 M HCl (which extracts all histones from undigested nuclei). Fig. 8 shows an electrophoretic analysis of these extraction products; the lower three tracks show HCl-extracted material. Five core histone bands are seen in undigested nuclei due to the presence of two H2B species in sea urchin sperm [20]. After trypsin digestion, all histones have been degraded and the fastest moving band observed has a mobility identical to that of peptide Gϕ1. Perchloric acid extraction (upper four tracks) yields only histone ϕ1 from undigested nuclei and from digested nuclei only a single peptide is extracted. This is likewise assumed to be Gϕ1 on the basis of its mobility. (Calibration of mobility was carried out with a sample of Gϕ1 obtained from tryptic digestion of ϕ1 in free solution as described above.)

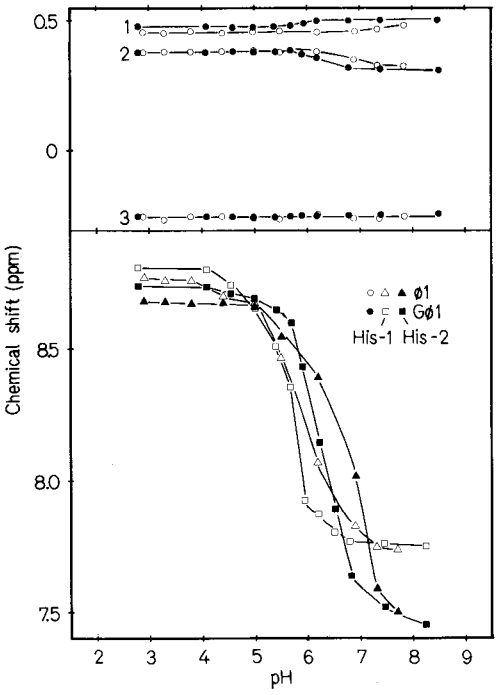

Fig. 7. *pH dependence of the chemical shift of the two histidine C-2 proton resonances and the three ring-current-shifted methyls (1, 2 and 3) in intact histone ϕ1 and in peptide Gϕ1*

This result indicates that the 82-residue region of histone ϕ1 that is more resistant to trypsin than the remainder is also resistant in chromatin. Thus the conformation of histone ϕ1 in free solution at high ionic strength is similar in overall features to that in chromatin.

CONCLUSIONS AND COMMENT

It has previously been demonstrated that both calf thymus histone H1 [6] and chicken erythrocyte H5 [7] consist of three structural domains in free solu-

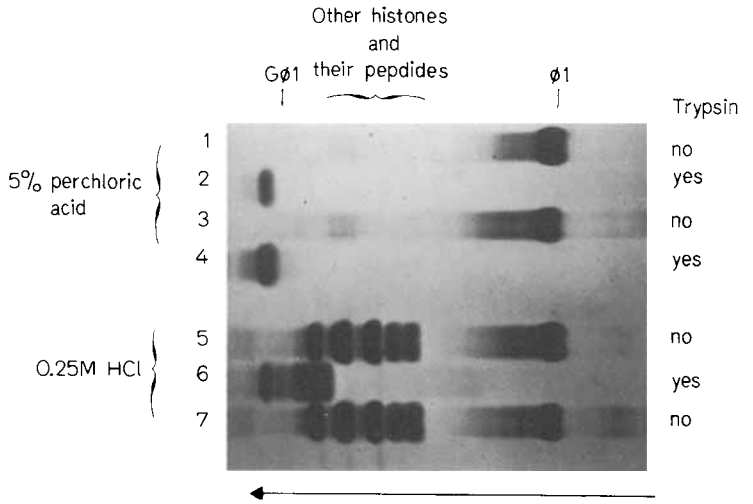

Fig. 8. *Dodecylsulphate gel electrophoresis of perchloric and hydrochloric acid extracts of trypsin-digested sperm nuclei*

tion. Histone ϕ1, although clearly an H1 histone, shows very considerable compositional and sequence differences from calf thymus H1 and from chicken erythrocyte H5 [10,15,20]. Nevertheless tryptic digestion yields a peptide of about 82 residues, similarly to H1 and H5. Although its position must await complete sequence data the structural homology between ϕ1 and histones H1 and H5 is clear, and the conclusion can be drawn that H1-type nuclear proteins consist of three structural domains.

A most important result of the present study is the finding that the same peptide Gϕ1 is released from nuclei as from ϕ1 in free solution at high ionic strength. Previous studies have involved only free solution digestions [6,7] and in the case of H5, digestion of a reconstituted complex of H5 and DNA [8]. It has always been a possibility that H1 was a fully folded conventional globular protein but extraction resulted in irreversible loss of structure in the N-terminal and C-terminal domains. It is now demonstrated that the preferential digestion of these flanking domains is observed in chromatin also, i.e. they are structurally quite distinct from the central globular domain. Whilst it follows from the present and earlier data that the central domain is globular it is not possible to draw firm conclusions regarding the conformation of the N-terminal and C-terminal domains in chromatin from this data. It is clear, however, that the results indicate an extended and exposed conformation for the terminal domains, and not a compact and buried one.

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P. Puigdomenech and J. Palau, Instituto de Biologia Fundamental, Universidad de Barcelona, Avenida San Antonio Maria Claret 171, Barcelona-26, Spain

C. Crane-Robinson, Biophysics Laboratories, Portsmouth Polytechnic, St Michael's Road, Portsmouth, Great Britain, PO1 2DT