

Preparation and characterization of histone H1 from the sperm of the sea-urchin *Sphaerechinus granularis*

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The separation and purification of histone H1 from the sperm of the sea-urchin *Sphaerechinus granularis* is described. Physical studies were used to compare this histone H1 molecule with H1 histones from other species. C.d. and 270 MHz n.m.r. spectroscopy indicate that, despite significant compositional differences from other sea-urchin sperm H1 histones, their secondary and tertiary structures are very similar. A large difference in helicity was, however, found between *S. granularis* histone H1 and calf thymus histone H1, and their n.m.r. and fluorescence spectra also differ considerably. It is concluded that secondary structure and tertiary structure have not been conserved in the evolution of the H1 histone family.

Nucleosomal coiling or folding induces the formation of a fibre of diameter about 20–35 nm (Ris & Kubai, 1970; Finch & Klug, 1976; Suau *et al.*, 1979), and it is generally assumed that histone H1 is responsible for the formation and maintenance of this structure *in vivo* (Littau *et al.*, 1965; Bradbury *et al.*, 1973; Finch & Klug, 1976; Renz *et al.*, 1977). Histone H1 consists of three structural domains. The central domain of approx. 80 residues is strongly conserved and serves to locate the molecules, whereas the flanking domains show much lower sequence conservation (Allan *et al.*, 1980).

Sea-urchin sperm contains histones bound to the DNA rather than protamine-like proteins, and the histone H1 molecule in this tissue differs from 'conventional' (e.g. calf thymus) histone H1 in having a high arginine content, more aromatic residues and histidine. Sequence comparison of the histone H1 molecule from the sperm of the sea-urchin *Parechinus angulosus* with that of calf thymus histone H1 shows the presence of considerable homology, but indicates that differences occur throughout the whole of the length of the molecule (Strickland *et al.*, 1980). Sperm is also a terminally differentiated tissue, and its histone H1 molecule shows homologies with histone H5 also, particularly with regard to the content and position of the arginine residues. The present paper describes the preparation and purification of histone H1 from the sperm of a sea-urchin not studied before. The

histone H1 molecule shows significant compositional differences. Physical measurements are used to look for similarities with and differences from other sea-urchin H1 histones and calf thymus histone H1. The structural approach to an understanding of the operation of histone H1 in chromatin is important in the absence of a functional assay of histone H1 action, i.e. it is hoped that, by the detailed analysis of structure in a wide range of histone H1 types, significant clues can be obtained as to their function and mode of action.

Experimental

Preparation of histone H1

Total histone was extracted from the sperm of the sea-urchin *Sphaerechinus granularis* by acid extraction by using the method of Geraci & Noviello (1979). Partial purification was obtained by chromatography on a Bio-Gel P-60 column (2.5 cm × 100 cm) at 4°C. Samples (300 mg) of total histone were loaded in 20 mM-HCl/50 mM-NaCl/6 M-urea and eluted by 20 mM-HCl/50 mM-NaCl (Van der Westhuyzen *et al.*, 1974) at a flow rate of 3–8 ml/h. Absorbance at 280 nm was used to monitor the elution pattern. Gel electrophoresis in 15% polyacrylamide containing 0.1% sodium dodecyl sulphate was used to check the purity of the eluted peaks. A large peak at the void volume contained aggregates of histones H3 and H4, and the histone H1

was found in the second peak at $V_e/V_0 = 1.50$ together with histone H2A. The last peak eluted was pure histone H2B₁.

Histone H1 was purified from the histone H1/H2A mixture by ion-exchange chromatography on a Whatman CM-52 CM-cellulose column (0.9 cm × 15 cm) equilibrated and eluted with 50 mM-sodium acetate (pH 4.5)/6 M-urea. A linear salt gradient of 0–0.5 M-NaCl was used to separate the two components. The histone H2A was eluted at 0.22 M-NaCl and the histone H1 at 0.36 M-NaCl. It is noteworthy that histone H1 is eluted after histone H2A, rather than before as is found with calf thymus histones. This is presumably due to the higher content of arginine residues in the sea-urchin histone H1.

Amino acid analysis

A 1 mg sample of protein was hydrolysed in 6 M-HCl for 20 h at 105°C, and multiple analyses were performed at the Institute of Organic Chemistry, Padova, Italy, on a JEOL JLC 6AH analyser.

Circular dichroism

C.d. spectra were obtained on Jouan II and Cary 61 dichrographs. The approximate concentration of stock solutions of protein was obtained by weighing freeze-dried protein, and the accurate concentration was measured from the tyrosine absorbance at 276 nm, assuming an absorption coefficient of $1340 \text{ cm}^{-1} \cdot (\text{mol of tyrosine})^{-1}$ and the presence of two tyrosine residues in a polypeptide chain of 250 residues. Each point in the c.d. spectra of Fig. 1 comes from measurement of a separate solution, adjusted to the desired molarity of NaCl. A single stock solution was used for the data presented in Fig. 1, but the results were checked with a second, independent, preparation of *S. granularis* histone H1.

Fluorescence

Fluorescence spectra were measured in quartz cylindrical cells of 0.5 cm diameter on a Perkin-Elmer MPF/3L spectrofluorimeter. Excitation was at 280 nm and emission was monitored at 305 nm. Emission intensity is reported as R_{Tyr} , the observed intensity with respect to that from a solution of tyrosine amino acid at the same concentration in 0.1 M-Tris/HCl buffer, pH 7.0 (Giancotti *et al.*, 1977). The solutions used for the fluorescence data of Fig. 1 were the same as those used for the c.d. measurements.

Electrophoresis

Electrophoretic separations were performed in 15% polyacrylamide gels (acrylamide/bisacrylamide ratio 66:1) essentially by method of Laemmli (1970)

with the modifications described by Thomas & Kornberg (1975).

Nuclear magnetic resonance

The 270 MHz n.m.r. spectra were obtained on a Bruker WH270 instrument, equipped with an Oxford Instruments 6.4 T superconducting magnet. Solutions of approx. 10 mg of protein/ml in 99.7% $^2\text{H}_2\text{O}$ in 5 mm tubes were referenced with respect to internal sodium 4,4-dimethylsilapentanesulphonate.

Results

Composition and molecular weight

Table 1 gives the amino acid analysis of *S. granularis* histone H1 together with those of two other sea-urchin histone H1 molecules, histone H1 from calf thymus and histone H5 of chicken erythrocytes. It is noteworthy that the arginine content of *S. granularis* histone H1 is even higher than those of the other two sea-urchin H1 histones given,

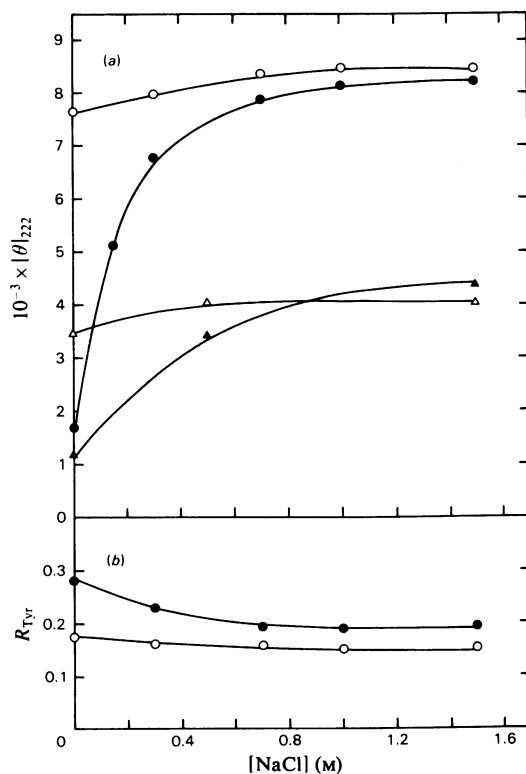


Fig. 1. C.d. (a) and fluorescence (b) data for *S. granularis* histone H1 at pH 3.5 (●) and at pH 7.5 (○) in 10 mM-sodium phosphate buffer as a function of ionic strength

C.d. data for calf thymus histone H1 are shown for comparison at pH 3.5 (▲) and at pH 7.5 (△).

Table 1. Amino acid analysis of H1 and H5 histones from different sources
Italicized values for S. granularis histone H1 are for residue contents showing significant differences from those of A. lixula and P. angulosus H1 histones.

	Amino acid composition (residues/100 residues)				
	Calf thymus histone H1 (Johns, 1971)	Chicken erythrocyte histone H5 (Greenaway & Murray, 1971)	Sea-urchin sperm histones H1		
			<i>Arbacia lixula</i> (Puigdomenech <i>et al.</i> , 1975)	<i>Parechinus angulosus</i> (Strickland <i>et al.</i> , 1976)	<i>Sphaerechinus granularis</i> (present work)
Asp (A)	2.5	1.8	2.2	1.8	1.5
Thr	5.6	3.3	2.5	1.9	4.0
Ser	5.6	13.0	6.7	6.0	7.6
Glu (A)	3.7	4.4	2.0	2.3	3.6
Pro	9.2	7.1	9.0	7.3	7.8
Gly	7.2	4.8	4.6	4.2	4.1
Ala	24.3	15.8	23.6	24.9	23.8
Val	5.4	3.9	2.8	3.7	3.1
Met	0	0.6	1.8	1.8	1.2
Ile	1.5	3.0	2.8	1.0	2.0
Leu	4.5	4.6	1.4	2.2	1.5
Tyr	0.4	1.7	0.8	0.9	0.8
Phe	0.5	0.6	0.4	0.4	0.5
Lys (B)	26.8	24.1	27.4	29.5	21.6
His (B)	0	1.4	1.2	1.0	0.9
Arg (B)	1.8	10.9	11.2	11.0	16.2
Acidic (A)	6.2	6.2	4.2	4.1	5.1
Basic (B)	28.6	36.4	39.8	41.5	38.7
B/A ratio	4.6	5.9	9.5	10.1	7.6
Lys/Arg ratio	15.0	2.2	2.4	2.7	1.3

and higher therefore than that of histone H5. There is a corresponding decrease in the lysine content of *S. granularis* histone H1 such that the total proportion of basic residues remains approximately constant.

Gel electrophoresis of *S. granularis* histone H1 together with histones H1 and H2B from calf thymus and cytochrome *c* as standards (Panyim & Chalkley, 1969) indicates a molecular weight of 24000, i.e. a molecule of about 220 residues. Preliminary sequence analysis of *S. granularis* histone H1 (W. N. Strickland & C. Von Holt, personal communication) by using CNBr cleavage indicates the presence of 3 methionine residues/molecule: on this basis the analysis of Table 1 indicates 2.0 tyrosine residues/molecule (the number observed in both *P. angulosus* and *A. lixula* H1 histones; Strickland *et al.*, 1980; Puigdomenech *et al.*, 1975) and a molecular weight of 27000, i.e. about 250 residues. Bearing in mind the problems of molecular-weight determination of histones by gel electrophoresis, the second, larger, molecular weight is to be preferred. This is in close agreement with the value of 248 residues determined for *P. angulosus* histone H1 (Strickland *et al.*, 1980) and considerably larger than rabbit histone H1 (213 residues; Cole, 1977) and chicken histone H5 (189 residues; Briand *et al.*, 1980).

Secondary structure

C.d. spectra of *S. granularis* histone H1 were obtained over a range of ionic strengths, and the ellipticity at 222 nm is plotted in Fig. 1 (circles) and compared with that of calf thymus histone H1 (triangles). Both show the increase of helicity typical for H1 histones to a maximum at about 1M-NaCl. On the basis of $[\theta]_{222} = -1000^\circ$ for random coil (Moss *et al.*, 1976) and -30000° for a helix (Chen *et al.*, 1974), the minimum value of -8400° represents 25% helix or 64 residues. With calf thymus histone H1, a minimum ellipticity of -4000° at 222 nm represents 10% helicity or about 23 residues of helix. The value for calf thymus histone H1 is close to that previously reported (Giancotti *et al.*, 1977), but that for *S. granularis* histone H1 is considerably greater than expected for a histone H1 molecule on the basis of homology of primary and secondary structure (Yaguchi *et al.*, 1977; Allan *et al.*, 1980). A check was therefore made by comparing the ellipticity at 222 nm of *S. granularis* histone H1, *A. lixula* histone H1 and chicken histone H5 in a single set of measurements on a second dichrograph. The value for *S. granularis* histone H1 was confirmed and that of *A. lixula* histone H1 found to be similar (-7900°), whereas that of the

histone H5 sample was -5000° , in agreement with that already published (Crane-Robinson *et al.*, 1976). It is therefore concluded that, although the H1 histone family shows considerable sequence homology, particularly in the folding domain (Allan *et al.*, 1980), the secondary structure content is not the same throughout.

Fluorescence spectroscopy

Fig. 1 also shows the change in intrinsic tyrosine fluorescence with ionic strength for *S. granularis* histone H1. At pH 3.5 the value of $R_{\text{Tyr}} = 0.28$ is typical for exposed tyrosine residues in the disordered protein (Cowgill, 1976; Giancotti *et al.*, 1977). On folding by salt addition there is a small decrease of fluorescence to $R_{\text{Tyr}} \approx 0.2$. If the two tyrosine residues of *S. granularis* histone H1 are homologous with those of *P. angulosus* histone H1, they are situated at positions 70 and 75, i.e. within the folding domain of the molecule (Strickland *et al.*,

1980; Allan *et al.*, 1980). The relatively small change in fluorescence intensity is thus not due to their remaining disordered, and must reflect their internal situation in the tertiary structure. A tyrosine residue at position 75 would be homologous to the single tyrosine residue of calf thymus histone H1, which is known to exhibit an increase of fluorescence intensity to $R_{\text{Tyr}} = 1.3$ (Giancotti *et al.*, 1977) as the ionic strength increases. It follows that the value of $R_{\text{Tyr}} = 0.2$ for *S. granularis* histone H1 in the folded form cannot be due to tyrosine-75 being a strong emitter, as in calf thymus histone H1, with tyrosine-70 having low or no emission. It follows that the tertiary structure around tyrosine-75 is not as in calf thymus histone H1. Although this conclusion on the non-identity of tertiary structure applies only to a limited region of the molecule, it is nevertheless in accord with the lack of secondary-structure conservation between the calf and sea-urchin histone H1 molecules.

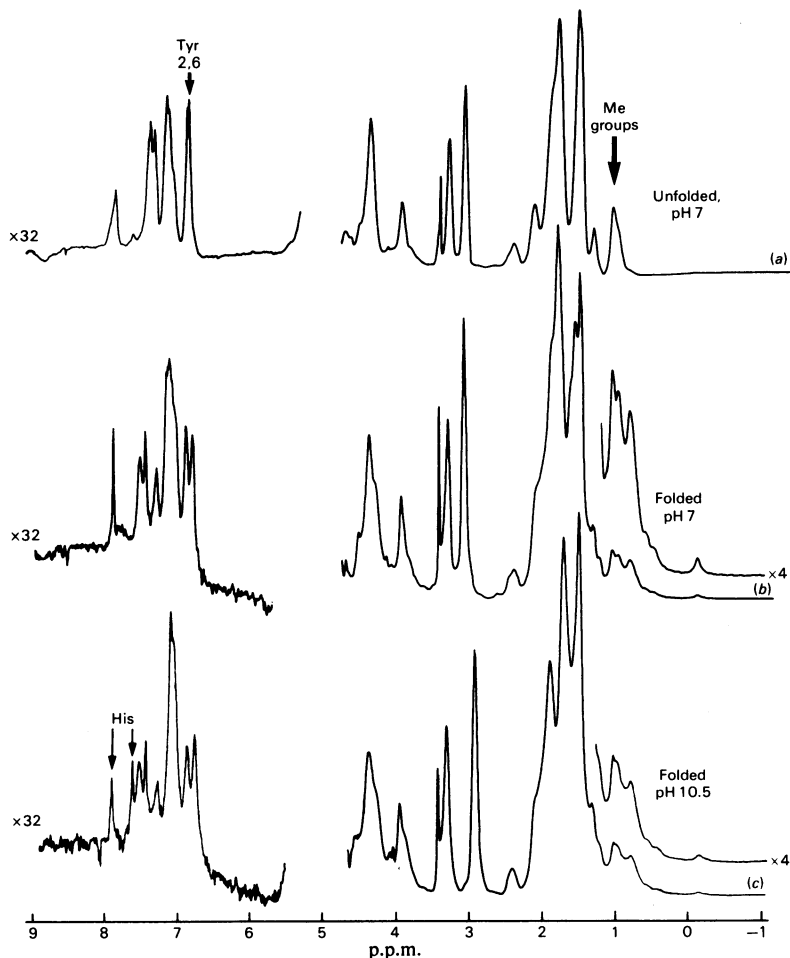


Fig. 2. 270 MHz n.m.r. spectra of *S. granularis* histone H1 (a) at pH 7.0 with no buffer or added salt (unfolded state) and (b) at pH 7.0 and (c) at pH 10.5 with added salt (folded state), ionic strength 1.0

Nuclear-magnetic-resonance spectroscopy

High-resolution n.m.r. spectroscopy was used to check the content of aromatic residues and to compare that tertiary structure of *S. granularis* histone H1 with that of *A. lixula* histone H1 and other H1 histones. The spectrum of *S. granularis* histone H1 at pH 7.0, in the absence of salt, is typical of a disordered protein with all residues of a single type having an identical chemical shift (see Fig. 2). In particular, the 34 methyl groups of the 17 residues of valine, leucine and isoleucine all resonate at essentially the same shift of 0.93 p.p.m. The resonance intensity of aromatic residues between 6.5 and 8.0 p.p.m. indicates that the phenylalanine:tyrosine:histidine molar proportions are probably 1:2:2. This is in agreement with the amino acid analysis shown in Table 1. On addition of 1 M-NaCl at pH 7.0, the spectrum of the upfield methyl groups and aromatic residues becomes complex, i.e. the protein folds. The tyrosine C-2,6 proton peak at 6.8 p.p.m. splits into two, confirming the presence of two tyrosine residues. At pH 10.5 there is further splitting of the histidine resonance, indicating the presence of two histidine residues. In general, the detail of the spectral perturbations in both the low-field and high-field regions is very similar to that already observed for *A. lixula* histone H1 (Puigdomenech *et al.*, 1980). Thus, although there are a significant number of compositional differences between the sperm H1 histones of these two sea-urchins, their tertiary structures are very similar. The aromatic and upfield methyl perturbations in the spectrum of calf thymus histone H1 are very different from those of the sea-urchin H1 histones. Although this appears to indicate differences of tertiary structure, this conclusion cannot be regarded as rigorous, since small changes in geometry and conservative replacements can give large spectral differences.

Discussion

The physical characterization described above indicates that both the secondary structure and tertiary structure of *S. granularis* histone H1 are very similar to those of *A. lixula* histone H1, despite significant compositional differences. The folding domain of H1 histones in which the secondary and tertiary structures are located is of approximately 80 residues (Hartman *et al.*, 1977; Puigdomenech *et al.*, 1980; Allan *et al.*, 1980) and is centrally located. Preliminary digestion studies indicate that *S. granularis* histone H1 also contains a trypsin-resistant domain of molecular weight approx. 9500, and is therefore similar in this respect to all the H1 histones so far studied (V. Giancotti, E. Russo, S. Cosimi & C. Crane-Robinson, unpublished work). It is concluded that this domain is essentially the same

in both sea-urchin sperm H1 histones. It is clearly a highly helical domain, and the present data indicate approx. 80% helix, which implies an 'all- α ' type of folding in the characterization of Levitt & Chothia (1976). Preliminary sequence findings (W. N. Strickland & C. Von Holt, personal communication) also indicate that most of the *N*-terminal domain is the same in *S. granularis* histone H1 and *P. angulosus* histone H1: the only difference in the first 26 residues of *S. granularis* histone H1 is the insertion of glycine between residues 17 and 18 of the *P. angulosus* histone H1 sequence (Strickland *et al.*, 1980). It follows that the increased arginine content of *S. granularis* histone H1 is located in the *C*-terminal domain of the molecule (presumably by replacement of lysine). This very basic region of histone H1 molecules does not fold in free solution (Hartman *et al.*, 1977), shows much sequence variation throughout the H1 histone family and is thought to be the main agent for the condensation of chromatin by H1 histones (Allan *et al.*, 1980).

Calf thymus histone H1, when folded at high ionic strength, shows a somewhat lower ellipticity than do the sea-urchin histones, a different n.m.r. spectrum and a different tyrosine fluorescence. Clearly there are significant differences in the folding domain of calf thymus histone H1 and the sea-urchin sperm H1 histones, despite the existence of a similar-sized globular domain in all these histones. The precise differences must await crystallographic study of these H1 histones.

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