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Secondary and tertiary structural differences between histone H1 molecules from calf thymus and sea-urchin (*Sphaerechinus granularis*) sperm

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Tryptic digestion of histone H1 from the sperm of the sea urchin *Sphaerechinus granularis* leaves a limiting peptide of approx. 80 residues that is of similar size to the limit peptide from calf thymus H1 or chicken erythrocyte H5. The *S. granularis* limit peptide folds to form tertiary structure similar to that of the intact parent histone H1 (shown by n.m.r. spectra), but the helical content is decreased by the digestion from 64 residues to 28. In contrast, intact calf thymus H1 and chicken erythrocyte H5 histones have only about 28 helical residues, which are preserved in their limit peptides. The extra helix in *S. granularis* is shown to be rapidly digested away by trypsin, and its location in histone H1 is discussed. A possible relationship of this structural feature to the length of linker DNA is proposed.

Tryptic digestion of the lysine-rich histone (H1) family of proteins results in a limit peptide of approx. 80 residues both in free solution (Hartman *et al.*, 1977; Aviles *et al.*, 1978) and in chromatin (Puigdomenech *et al.*, 1980; Allan *et al.*, 1980). In the histone H1 molecules so far studied, no structural distinctions have been found between different members of the family in that the limit peptides were all of closely related size and corresponded to the same segment of the polypeptide chains when these were aligned for maximum homology (Allan *et al.*, 1980). Moreover, it was found that the number of helical residues in the limit peptides appeared to be the same as in the parent molecules (from the c.d. spectra), and the tertiary folding was entirely confined to the protected peptides. Although these results strongly emphasized homologies between histone H1 molecules and pointed to structural (and by implication, functional) similarities, they did not offer any explanations of how the H1-family molecules characteristic of repressed genomes, e.g. chicken erythrocyte H5 and sea-urchin sperm H1, might differ. Recent results on histone $H1^0$, associated with the quiescent G_0 phase of the cell cycle, likewise fit into this pattern of uniformity (P. D. Cary, M. Hines & E. M. Bradbury, unpublished work). We have published a paper (Giancotti *et al.*, 1981) pointing out that histone H1 from the spermatozoa of the sea urchin *Sphaerechinus granularis* contains about twice as many helical residues as calf-thymus H1 (approx. 64, compared with approx. 23). If 64 helical residues were confined to a folded domain of approx. 80 residues, the helicity would be exceptionally high and slightly in excess even of that in haemoglobin. A lack of structural homology between sea-urchin and calf histones H1 was clearly implied. The results presented here show that *S. granularis* histone H1 contains a helical region of about 36 residues that is very rapidly digested by trypsin and so does not appear in the limit peptide. The *S. granularis* limit peptide is only about four residues shorter than that from calf thymus histone H1 and is therefore of similar helicity. This structural difference between the two molecules could be the basis of a functional distinction related to an ability of the sea-urchin spermatozoa histone H1 totally to repress the organism's genome.

Abbreviations used: Tos-Lys-CH_2Cl, 7-amino-1-chloro-3-L-tosylamidoheptan-2-one ('TLCK'); SDS, sodium dodecyl sulphate.

Experimental

Calf thymus histone H1 was extracted by the procedure of Johns (1964) and purified by exclusion chromatography on Bio-Gel P-60 in 10mM-HCl and ion-exchange on Bio-Rex 70 eluted with a gradient of 0.75–1.25M-NaCl in 10mM-HCl. Histone H1 from the sperm of *S. granularis* was extracted and purified as described by Giancotti *et al.* (1981). For trypsin digestion, the H1 histones (approx. 3mg/ml) were dissolved in 20mM-Tris/HCl, pH8, with NaCl

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added to 1.5 M to ensure complete folding of the histones H1 (Bradbury *et al.*, 1975). Trypsin was added in a ratio of 1:1000 (w/w). Digestion was stopped by the addition of Tos-Lys-CH_2Cl in a ratio of 1:2 (enzyme/inhibitor, w/w) and then adjusting the pH to 2 with HCl. For electrophoretic analysis the reaction mixture was diluted 1:1 with 2% (v/v) SDS/30% (w/v) sucrose in 0.3 M-Tris/HCl, pH 8.8.

The limit peptides from both calf thymus and *S. granularis* histone H1 were purified by exclusion chromatography on Bio-Gel P-10 or P-30 (2.5 cm × 100 cm columns) in 20 mM-HCl/50 mM-NaCl as described by Böhm *et al.* (1973). The purified peptides were recovered by freeze-drying after dialysis. The molecular weights and purities of the peptides were studied by electrophoresis on 0.1% SDS/20% (w/v) polyacrylamide gels (21 cm long, 1.5 mm wide) calibrated with cytochrome *c* and ubiquitin. C.d. spectra were obtained with a Cary 61 dichrograph by using a cell of 0.2 cm path length. The protein concentrations were determined by measuring the tyrosine absorbance at 270 nm, by using an absorption coefficient of 1340 litre·mol^{-1}·cm^{-1} and assuming 2 mol of tyrosine/mol of calf thymus histone H1 and its limit peptide. The molecular weight of the peptides used was that obtained with the 20%-polyacrylamide gel. C.d. spectra were run in KF solution for maximum transmittance. Amino acid analyses were obtained by 20 h hydrolysis at 105°C in 6 M-HCl.

Results and discussion

Fig. 1(*a*) compares the time course of trypsin digestion of histone H1 from calf thymus and sea-urchin sperm in free solution. In each case a limit peptide (LP) is produced, and these have both been purified by exclusion chromatography on Bio-Gel P10 as detailed in the Experimental section. Purified samples were compared by SDS/polyacrylamide-gel electrophoresis in a 20% gel calibrated with cytochrome *c* and ubiquitin (Fig. 1*b*). Both samples appear pure, and their molecular weights were estimated from the gel as 9730 for the calf thymus peptide (LP-H1-CT) and 9290 for the *S. granularis*

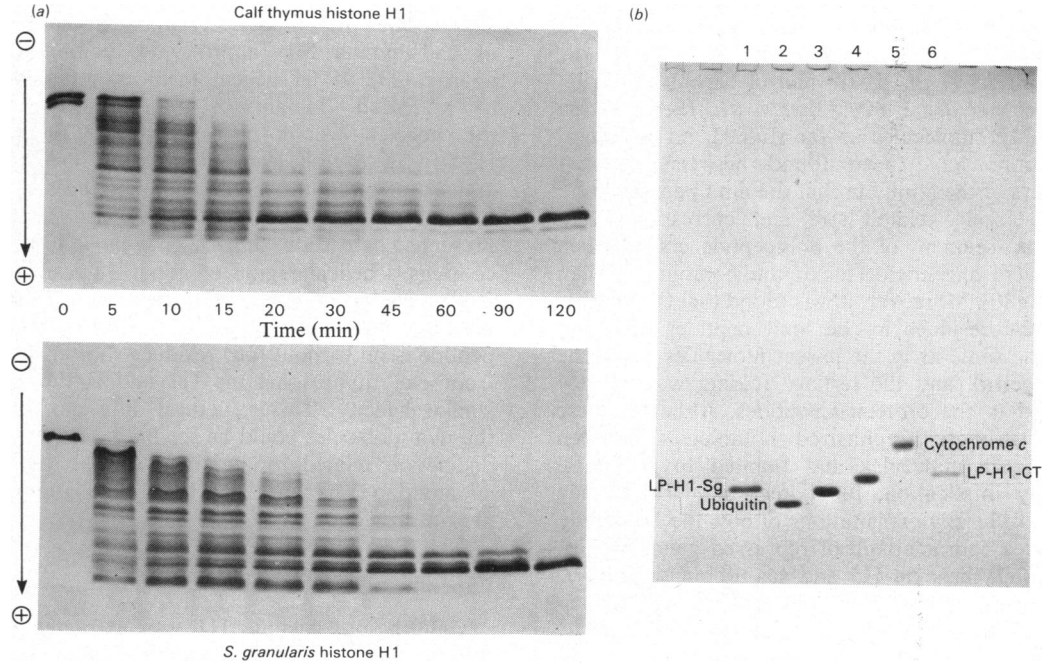

Fig. 1. (*a*) *Time course of trypsin digestion of histone H1 from S. granularis and calf thymus, and* (*b*) *purity and molecular-weight estimation of purified limit peptides from histone H1 of calf thymus* (*LP-H1-CT*) *and of S. granularis sperm* (*LP-H1-Sg*)

(*a*) Electrophoresis was in 15% (w/v) polyacrylamide (acrylamide/bisacrylamide, 66:1) gels containing 0.1% SDS. (*b*) Electrophoresis was in 20% polyacrylamide (acrylamide/bisacrylamide ratio, 66:1)/0.1% SDS, gels. Lanes 1 and 3, LP-H1-Sg at loadings ×1 and ×5; 6 and 4, LP-H1-CT at loadings ×1 and ×5; 2, ubiquitin (mol.wt. 8450); 5, cytochrome *c* (mol.wt. 11 700).

peptide (LP-H1-Sg). This suggests that the *S. granularis* peptide is some four residues shorter than the calf peptide.

Table 1 gives the amino acid composition of both peptides, together with that of another sea urchin (*Arbacia lixula*), two other published analyses of the peptide from calf thymus and that derived from histone H1 of the fruitfly *Ceratitis capitata*. Good agreement is noted with previous data for calf thymus, but significant differences between the two sea-urchin peptides are evident. These differences reflect, in part, the differences previously noted in the composition of the intact sea-urchin proteins (Giancotti *et al.*, 1981). The two sea-urchin peptides are identical as regards the conserved residues, i.e. two histidine, two tyrosine and one phenylalanine.

To test whether the calf peptide is the same as that described by Hartman *et al.* (1977) and how the sea-urchin peptide compares with it structurally, their c.d. spectra were obtained at high ionic strength [an essential condition for renaturing histone H1 molecules (Bradbury *et al.*, 1975)]. Fig. 2(*a*) shows the results, together with spectra recorded for the parent histones. By using the ellipticity at 222nm as a guide to the helix content [−1000° for random coil (Moss *et al.*, 1976) and −30000° for a complete helix (Chen *et al.*, 1974)], we calculate the numbers of helical residues shown in Table 2. In these calculations the ellipticity has been partitioned solely between helix and random coil, since there is no indication of β-structures either from the c.d. curves or from i.r. spectra of histones H1 (Bradbury *et al.*, 1975).

The helicity of the intact calf histone H1 molecule is in good agreement with previously published values (Smerdon & Isenberg, 1976; Giancotti *et al.*, 1977), whereas that of intact *S. granularis* histone H1 agrees with that reported by Giancotti *et al.* (1981) but is somewhat at variance with that reported for histone H1 from the sperm of *A. lixula* (Puigdomenech *et al.*, 1980). The striking feature of the results is that although intact calf histone H1 and both peptides contain about the same number of helical residues (which is close to the number found for intact histone H5 and its tryptic peptide; Aviles *et al.*, 1978), the intact *S. granularis* histone H1 molecule contains significantly more helix. Tryptic digestion has clearly removed a helical section from the *S. granularis* histone H1 molecule to leave a peptide having similar size and helicity to the peptide from calf thymus histone H1.

To investigate this additional structural feature of the sea-urchin molecule, the time course of trypsin digestion was monitored for both proteins

Table 1. *Amino acid analyses of limit peptides (LP) of H1 histones from different sources*

Sources of data: (a) Hartman *et al.* (1977); (b) Barbero *et al.* (1980); (c) the present work; (d) Puigdomenech *et al.* (1980). Abbreviations used: GH1, globular peptide from calf thymus histone H1; TRC, trypsin-resistant cores from calf thymus and *C. capitata*; LP-H1-CT, limit peptide from histone H1 of calf thymus; LP-H1-Sg, limit peptide from histone H1 of *S. granularis* sperm; GΦ1, globular peptide from sea urchin (*A. lixula*) sperm histone H1 (known as Φ1).

	Amino acid composition (mol/100 mol)					
	Calf thymus			Sea urchins		Fruitfly
	(a) (GH1)	(b) (TRC)	(c) (LP-H1-CT)	*S. granularis* (c) (LP-H1-Sg)	*A. lixula* (d) (GΦ1)	*C. capitata* (b) (TRC)
Asp	5.4	6.1	5.1	4.0	5.0	5.9
Thr	6.0	5.5	5.8	7.5	5.8	6.8
Ser	11.8	10.6	11.1	7.5	5.4	10.6
Glu	6.6	7.6	5.6	5.3	4.1	6.8
Pro	3.7	4.7	3.3	4.8	5.9	5.9
Gly	12.7	11.7	10.6	9.2	12.1	6.2
Ala	17.4	16.2	18.0	16.3	13.5	16.5
Cys	0.0	0.0	0.0	0.0	N.D.	0.7
Val	5.9	6.7	6.2	8.7	7.1	6.7
Met	0.0	0.0	0.0	3.6	5.2	0.7
Ile	1.9	2.3	1.7	5.3	8.2	5.6
Leu	9.7	9.5	9.9	4.0	3.6	6.6
Tyr	1.1	1.0	1.0	2.3	2.5	2.0
Phe	1.1	1.4	1.1	1.4	1.4	2.0
Lys	14.6	14.4	18.4	7.5	11.8	13.2
His	0.0	0.0	0.0	2.4	2.3	1.1
Arg	2.1	2.4	2.3	10.2	6.1	2.5

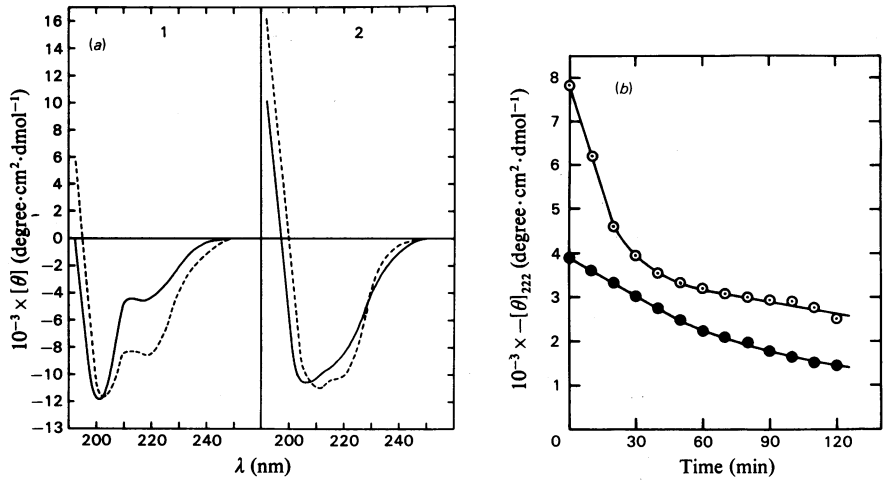

Fig. 2. (*a*) *C.d. spectra of intact calf thymus histone H1 and its limit peptide LP-H1-CT and intact S. granularis histone H1 and its limit peptide LP-H1-Sg, and* (*b*) *time course of trypsin digestion of histone H1 from calf thymus and S. granularis sperm monitored by the ellipticity at 222 nm*

(*a*) Panel 1, intact molecules (——, calf; – – – –, sea urchin); panel 2, limit peptides (——, calf; – – – –, sea urchin). (*b*) ●, calf thymus; ⊙, *S. granularis* sperm.

Table 2. *Helicity of calf and sea-urchin H1 histones and their tryptic peptides*

	θ_{222} (degree·cm^{-2}·dmol^{-1})	Helix (%)	No. of residues in chain	No. of helical residues
Intact calf histone H1	−4750	12.9	214	28
Intact *S. granularis* histone H1	−8700	26.6	240	64
Peptide LP-H1-CT	−9000	27.6	88	24
Peptide LP-H1-Sg	−10600	33.1	85	28

in a dichrograph. Fig. 2(*b*) shows the time-dependence of the ellipticity at 222 nm as a measure of loss of secondary structure. Over 120 min, the helicity of the calf histone H1 sample decreases approximately linearly with time. Since the purified calf thymus peptide contains the same number of helical residues as intact calf histone H1, this decrease in helicity with time is due to a slow degradation of the globular domain taking place. For the sea-urchin histone H1, the same slow degradation is observed, which extrapolates back to an ellipticity of approx. −4000° at zero time (as for calf histone H1), but this is preceded by a rapid decrease in ellipticity in the first 30 min. This rapid decrease, from −8000° to −4000°, clearly represents the extra helicity in the sea-urchin molecule (about 36 residues) and this extra helix is clearly very susceptible to trypsin digestion. It might be that removal of the extra residues from sea-urchin histone H1 also leads to breakdown of the tertiary structure. The n.m.r. spectrum of the limit sea-urchin peptide LP-H1-Sg was therefore obtained at high ionic strength and compared with that of the intact molecule at equimolar concentration (0.3 mM) (see Fig. 3). There is a considerable decrease in the resonance from lysine and arginine residues in the peptide, as expected from its amino acid composition (Table 1). The identity of the chemical shifts of the hydrophobic residues valine, leucine and isoleucine in peptide and parent molecule indicates that the peptide folds like the intact sea-urchin sperm histone H1 molecule. This identity is seen in a general way from the 27 or so overlapping methyl groups that resonate between 1.2 and 0.6 p.p.m. and exhibit only small perturbations from a random-coil position of approx. 0.95 p.p.m. The identity of the three ring-current-shifted (single) methyl groups at 0.5, 0.4 and −0.3 p.p.m. is, however, a striking demonstration that the peptide has the same tertiary structure as the intact parent histone H1.

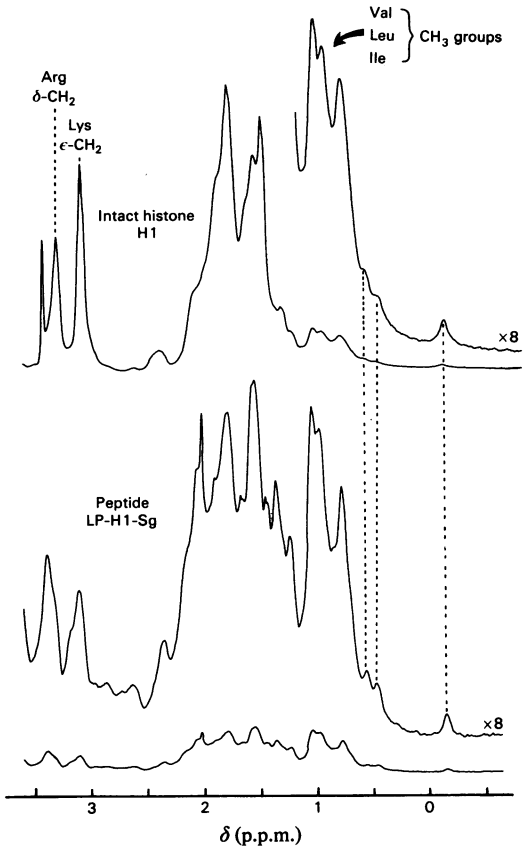

Fig. 3. *Proton spectra (270MHz) of intact S. granularis sperm histone H1 and its limit peptide LP-H1-Sg*
Three upfield ring-current-shifted methyl peaks are defined by vertical lines. Vertical expansions ×8.

The present data therefore define the presence of an additional, trypsin-sensitive, helical element in sea-urchin histone H1 above that found in calf histone or chicken erythrocyte histone H5. Where could this extra helical element be located in the molecule? Since the gel of Fig. 1(*a*) indicates the presence of several peptides both larger and smaller than LP-H1-Sg that are largely degraded by about 30min (when this helical element has been lost), one cannot at present decide whether the helical element is well separated in the sequence from the protected peptide or close to it. It must also be borne in mind that the helix could be the terminus of the folding domain in the intact molecule and located on the surface such that it is readily excised by trypsin. Its absence might not prevent the remainder of the folding domain from taking up its native structure and exhibiting the same perturbations in the n.m.r. spectrum (normally only from deeply buried residues). Only a structural study of several peptides from sea-urchin histone H1 will allow those possibilities to be resolved.

We do not think that there are any major differences between the histone H1 molecules of *A. lixula* and *S. granularis*. A previous estimate (Puigdomenech *et al.*, 1980) of the helicity of intact *A. lixula* sperm histone H1 was in error, and it is clear from re-measurement (Giancotti *et al.*, 1981) that this histone does contain the additional helical element.

Sea-urchin sperm chromatin has the longest DNA repeat length (240 base-pairs) so far observed (Spadafora *et al.*, 1976), and the length of linker DNA may be related to the type of histone H1 molecule present (Noll, 1976). If this is so, the question arises as to what feature of histone H1 structure is involved. It is probably not due to the overall arginine content, since erythrocyte chromatin containing histone H5 has a repeat of 212 base-pairs (Weintraub, 1978), and the arginine content is much the same as in sea-urchin sperm histone H1 (approx. 11%). A second possibility is the length of the histone H1 molecules, since those of sea-urchin sperm have about 250 amino acids, compared with 210–215 in calf thymus. Again, if histone H5 is any guide, this does not seem likely, since chicken erythrocyte H5 has only 189 residues, i.e. shorter than calf histone H1, whereas the erythrocyte chromatin repeat is intermediate between those of calf thymus and sea-urchin sperm. A third possibility raised by the present observations is that the additional helical element in sea-urchin sperm histone H1 is related to the increased DNA repeat length.

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