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The chromatin of sea urchin sperm

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Digestion of sea urchin sperm nuclei with micrococcal nuclease yields nucleosomal monomer fragments of 151 and 164 base pairs. Prior trypsin treatment of the sperm chromatin does not alter the size of these monomer DNA fragments despite the fact that the H1 histone is reduced to a limit globular peptide of about 83 residues. Heterologous reconstitution experiments show that this peptide is capable of protecting an extra 22 base pairs beyond the core particle in a chromatosome. Nuclease digestion of reconstitutes from DNA and sperm core histones yields a core monomer of about 141 base pairs. It is concluded that this sperm chromatin contains a chromatosome of 164 bp essentially similar to that observed in the more usual chromatins. Edman degradation of the H1 limit peptide shows its sequence to be closely analogous to the corresponding peptide of calf H1 and chicken H5. Circular dichroism studies of histone H1 from the sperm of three sea urchin species demonstrate the presence of trypsin-sensitive helical regions outside the globular domain that are absent in calf H1 and chicken H5.

Introduction

The chromatin of sea urchin sperm contains a full complement of histones, contains no protamine-like molecules and has the longest DNA repeat length, 241 bp, so far measured [1]. It also has a very long H1 molecule of about 250 amino acids [2–4], and so it was proposed [5,6] that these two features are related in that H1, as the 'linker' histone, needs to be longer when a greater amount of linker DNA must be controlled in the chromatin. Such a simple correlation cannot be the whole story, however, since chicken erythrocyte chromatin, with a somewhat larger than normal repeat (212 bp [6]), contains large amounts of histone H5 which is a rather short H1-type (189 residues [7]). Factors other than length are clearly involved, such as the amino acid composition of the H1: longer DNA repeat lengths are typically associated with H1 histones containing increased

Abbreviation: PMSF, phenylmethylsulphonyl fluoride.

Data supplementary to this article are deposited with, and can be obtained from: Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/354/91643/908 (1987) 70. The supplementary information includes: Automated Edman degradation of the globular fragment from *Arbacia lixula* sperm histone H1.

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amounts of arginine, e.g., chicken erythrocyte and sea urchin sperm. The amount of H1-type histones present may also be critical, e.g., chicken erythrocyte chromatin has 1.3-times the amount of linker histone as rat liver chromatin [8].

In some histone-containing sperm chromatins the linker H1-type histone is accompanied by protamine-like molecules. For example in *Ensis minor,* a bivalve mollusc, there is a very large H1 species and also a protamine-like molecule, but the DNA repeat of 200 bp is that typical for mammalian somatic cells [9]. In the sea worm *Platynereis dumerilii*, the sperm contains two very short H1 species (about 120 amino acids [10]), a small amount of a fairly normal length H1 and some protamine-like molecules [11], yet the repeat length is quite short (about 165 bp (Sellos, D., personal communication). The situation in these sperm chromatins is clearly complex and no simple correlations between repeat length and linker-histones can at present be made. Additionally, the core histones can show significant variation; in sea urchin sperm the H2B species have very extended N-terminal domains [13], whilst the remaining three core histones do not differ greatly from those of calf thymus. The extent to which an 'altered' core histone might influence the chromatin structure is unknown, although studies of calf/sea urchin interspecies core histone complexes indicate that such complexes are probably not affected by extended N-domains [14]. The histone-containing chromatin of sperm is clearly a complex system that needs further study, not only for its own sake, but for the light it may throw on the structure of somatic chromatin. Sea urchin sperm chromatin does not have the added complexity of further proteins beyond the normal five histones and so is an appropriate material to study. It has, moreover, been used as an example of a long repeat length chromatin in comparisons with calf thymus and other chromatins [15]. A very recent study of sea urchin sperm chromatin using electron microscopy and X-ray diffraction indicates that 30 nm filaments are present that are very similar to those of chicken erythrocyte chromatin [39]. If the higher-order structure of sea urchin sperm chromatin is very close to that of somatic cell chromatin, it is to be expected that the essential features of nucleosome structure would also be found in such long repeat material.

Keichline and Wassarman [16] reported that DNAase I digests of sea urchin sperm chromatin show strong fragments of 70 and 80 nucleotides, suggesting the presence of a typical 80 bp/turn core particle. They also observed a shoulder at about 140 bp and a broad peak in the region of 160 bp in micrococcal nuclease digests that might indicate the existence of normal core and chromatosome units. The present study was undertaken therefore firstly to establish whether sea urchin sperm chromatin contains the usual type of core particle and chromatosome, and for the latter whether this is maintained by the unusually long histone H1 in a manner similar to that in somatic chromatins [17,18]. The structure of the sperm-specific H1 histone has also been investigated further, since it appears to show marked conformational differences from somatic H1 species. Three different sea urchins have been used in the present study, *Arbacia lixula, Sphaerechinus granularis* and *Parechinus angulosus* and no essential differences between them have been found.

Materials and Methods

Nuclei and chromatin

Sea urchin sperm cells, either fresh or frozen in 50% glycerol at −20°C were washed in filtered sea water and then homogenized in a Potter-Elvejhem homogeniser in 0.34 M sucrose/60 mM KCl/0.15 mM spermine/0.5 mM spermidine/0.4 mM PMSF/15 mM Tris-HCl (pH 7.4)/0.5% Nonidet P-40, until they appeared as pure nuclei in the light microscope. The nuclei were then resuspended in the same buffer without detergent as about 1 mg/ml DNA. Digestions with micrococcal nuclease (1500 units/mg, Boehringer) at 37°C were carried out in the same buffer with a total effective concentration of 1 mM $CaCl_2$, employing 120 units of enzyme per mg of DNA. The reaction was stopped by adding 2.5 mM EDTA at the appropriate time. The DNA was deproteinized by proteinase K digestion and phenol/chloroform treatment. When trypsin digestion was involved, the nuclei were previously digested with micrococcal nuclease at 4°C using 60 units nuclease per mg of DNA for 30 min in order to avoid excessive increase of viscosity during proteinase treatment.

Trypsin digestion was carried out in the absence of PMSF at 37°C with a weight ratio 1 : 75 trypsin/DNA. When nuclease digestion followed trypsinization a 3 : 1 excess of trypsin inhibitor was added together with 1 μg/mg TLCK. Typically 1 ml samples were studied (1 mg/ml DNA) and the percentage of acid-soluble nucleotides depended on the extent of digestion, amounting to a maximum of 70% in trypsin-treated samples. The final DNA analysed represented between 10 and 20% of the total DNA.

Nucleosome reconstitution

Total histones were prepared by adding an equal volume of 4 M NaCl to a suspension of nuclei followed by centrifugation at 40000 rpm for 1 h in a Beckman SW60 Ti rotor. Core histones were prepared by extraction of nuclei with 0.25 M HCl after treatment with 5% perchloric acid to extract H1. DNA (salmon testes, Sigma Type III, purified by phenol/chloroform treatment) was dissolved in 0.1 mM EDTA/10 mM Tris-HCl (pH 7.4). Both histone and DNA solutions were dialysed against 2 M NaCl/0.1 mM PMSF/0.2 mM EDTA/10 mM Tris-HCl (pH 7.4), mixed at an approximate 2 : 1 DNA/histone ratio by weight and then brought to 0.2 M NaCl/0.1 mM PMSF/10 mM Tris-HCl (pH 7.4) by step dialysis. The reconstituted material was digested in this buffer at 37°C with 1 mM $CaCl_2$ added using 100 units of nuclease/mg DNA.

Electrophoresis

Chromatin samples after trypsin digestion were either extracted with 5% perchloric acid or precipitated with 20% trichloroacetic acid for electrophoresis of histone H1 or total histone peptides as described [19]. Protein gels were in 18% polyacrylamide according to Laemmli [20]. Electrophoresis of duplex DNA was either in 10% polyacrylamide gels using a Tris-borate-EDTA buffer [21] or in 7% acrylamide gels using Tris-phosphate buffer [25]. DNA calibration was carried out with pBR322 DNA digested with either *Hae*III or *Hpa*II.

Histone H1 preparation

Sperm nuclei prepared as above were extracted with 5% perchloric acid and the H1 purified using an NaCl gradient with Whatman CM52 in 50 mM sodium acetate buffer/6 M urea, as in Ref. 22. The limit trypsin fragments of H1, designated GH1, were prepared from *A. lixula* and *S. granularis* H1 by digestion in free solution in 20 mM Tris-HCl (pH 8) with 1.5 NaCl added and a H1/trypsin ratio of 1000. Digestion was stopped by the addition of TLCK and the peptides were purified by exclusion chromatography on Bio-Gel P10 in 20 mM HCl/50 mM NaCl [23] or on Sephadex G-50 in 10 mM HCl/0.5 M NaCl [24].

H1 reconstitution

H1/H5-depleted chicken erythrocyte polynucleosomes having an unchanged repeat length were prepared at 0.1 mg/ml DNA in 80 mM NaCl/5 mM Tris-HCl/0.1 mM EDTA (pH 7.5) as in Ref. 25. Intact H1 molecules and GH1 from *S. granularis* were reconstituted by addition in 80 mM NaCl at a molar ratio, r, of histone to nucleosome of unity. Trypsin treatment of chicken erythrocyte polynucleosomes and reconstitutes was with TPCK-trypsin at a final concentration of 0.2 μg/ml for 5 min at 0°C. The reaction was stopped with 1 μg/ml of soya bean trypsin inhibitor. Subsequent micrococcal nuclease digestions were carried out at 1.0 A units/ml using 20 units enzyme/ml at 37°C in the same medium with 1 mM $CaCl_2$ added.

Sequencing

Automated Edman degradations were carried out in a Beckman 890C sequencer using a dimethylallylamine programme in the presence of polybrene [26]. Phenyl thiohydantoin amino acids were identified by high-pressure liquid chromatography as in Ref. 27.

Circular Dichroism

Spectra were obtained using a Jasco J500A dichrograph. The solvent was 10 mM sodium phosphate (pH 8)/1.6 M NaCl for the helicity measurements given in Table I. Potassium fluoride was substituted for NaCl in the folding experiments of Fig. 7B. The trypsin digestion time-course experiments were carried out at a histone/trypsin ratio of 1000 : 1 in the phosphate-buffered saline medium. Histone concentrations were determined by the extinction at 275 nm, assuming 1340 per mol tyrosine and the presence of two tyrosine residues per chain of 250 amino acids.

Results and Discussion

Digestion of nuclei

Fig. 1 shows a time-course of micrococcal nuclease digestion of *A. lixula* sperm nuclei analysed on a double-stranded DNA gel. After 5 min digestion the expected approx. 241 bp repeat is seen and the monomer shows a sharp front edge at approx. 150 bp. With progressive digestion, intensity increases in the monomer region of the gel with a number of defined components being visible, including for example a subnucleosomal component of 125 bp. There is no clear indication of either core (146 bp) or chromatosome (168 bp) length DNA fragments despite the use of 37°C to promote the trimming action of the nuclease. A possible explanation for the lack of the expected DNA fragments is that the chromatin remains highly condensed, despite a multitude of cuts, with the result that trimming action is inhibited. It was in fact observed that turbidity increases during the digestion with the formation of some precipitate at longer digestion times. It is worth noting that other authors have observed that chromatosome-length DNA is less readily obtained from nuclease digests of nuclei than from chromatin [28]. A second approach was therefore tried in which the first digestion was at 4°C in sperm nuclei followed by chromatin extraction in 0.2 mM EDTA with blending. The chromatin was then re-digested at 37°C to promote trimming. The resulting 'monomer' DNA consisted of several components on a strong background, much as in Fig. 1. Although the substrate for this second approach was an extracted chromatin, the micrococcal nuclease required 1 mM divalent cations and this strongly promotes nucleosomal condensation and aggregation.

A method of disaggregating chromatin is to use mild trypsin treatment, since this removes (1) the N- and C-domains of histone H1, leaving just the globular doman [24,18], and (2) the N-domains of the core histones [29]. It has been shown that, following trypsinisation of chicken erythrocyte chromatin, micrococcal nuclease is able to define the chromatosome DNA length [19]. Fig. 2a shows a double-stranded DNA gel of nuclei digested for 20 min at 37°C with micrococcal nuclease after prior digestion with trypsin at 4°C for 0 to 180

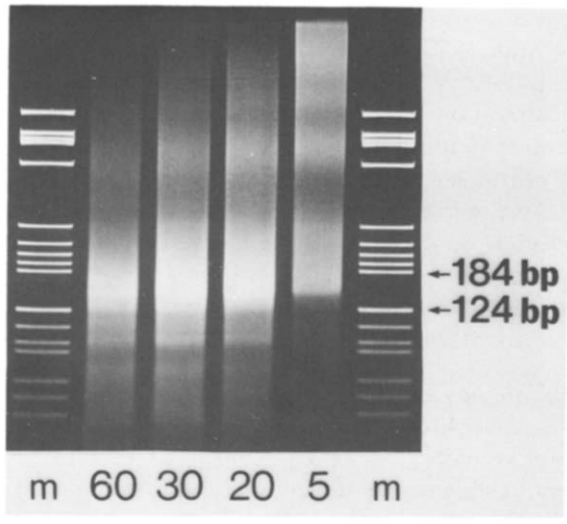

Fig. 1. Time-course, 5 to 60 min, of micrococcal nuclease digestion at 37°C of *A. lixula* sperm nuclei. 200 units nuclease/mg DNA. m, *Hae*III digest of pBR322 DNA.

min. The trypsin digestion causes gradual rupture of the nuclei such that beyond about 10 min trypsinisation the nuclease digestion is in reality on chromatin. After about 60 min trypsinisation, nuclease digestion produced monomer-length DNA, which remained up to 180 min. To determine the fragment lengths, an aliquot of the 180 min trypsin/20 min nuclease digest was co-electrophoresed with the marker DNA in the final lane. Fig. 2b shows a scan of this lane: two bands of 164 and 151 bp are observed. When lane M is compared (Fig. 2b) with a scan of lane 0′ (no prior trypsinisation) it is apparent that the same fragment lengths are also present in the 'nuclease-alone' digest, albeit much broader. The 164 bp band is a little shorter than the typical chromatosome but presumably indicates the presence of a two-superhelical turn structure. The 151 bp fragment is well above the usual core particle size, however.

Core particle length DNA is well defined in digests of chromatin that have lost H1, so that trimming proceeds readily down to about 146 bp. What then is the state of the histones in the sea urchin chromatin following trypsin treatment? Fig. 3 shows an SDS gel of (A) proteins extracted by 5% perchloric acid from the chromatin followed

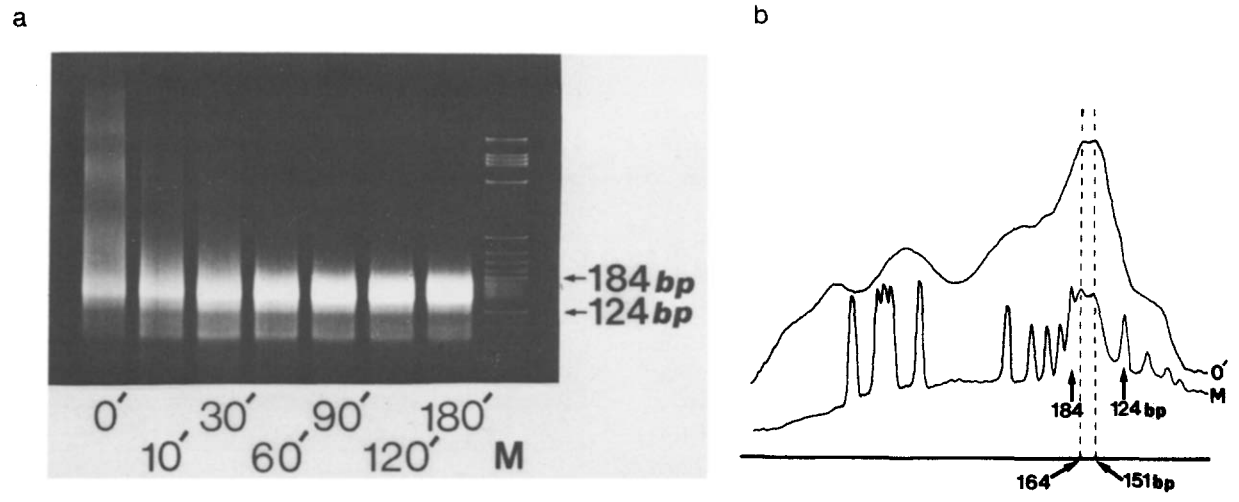

Fig. 2. (a) Double-stranded DNA gel of *A. lixula* nuclei digested with 200 units of micrococcal nuclease/mg DNA for 20 min at 37°C following various times of prior trypsin digestion at 4°C. All lanes contain equal loadings of DNA except M, which contains a reduced quantity of the 180 min trypsin digest together with marker DNA (*Hae*III/pBR322). (b) Densitometry of (a). O′ is the lane containing products having no trypsin treatment. M is the lane of 180 min trypsin treatment plus marker. DNA length in base pairs.

by trichloroacetic acid precipitation, i.e., H1 and its degradation products, and (B) total histone precipitated by trichloroacetic acid. It is clear that at the earlier times of trypsin digestion, e.g., 30 min, all the H1 is digested to two fragments, the smaller of which, GH1, is the 'limit' product of digestion defined previously and consisting of about 82 residues [24]. At this time the core histones are also nearly completely degraded to their 'limit' fragments. It is surprising, therefore, that with this level of histone digestion, a core particle length is not seen at all. This could be due to very tight binding of GH1, or even of fragments from the N- and C-domain of H1 that inhibit nuclease trimming.

Nucleosomal reconstitution

Core particles. In order to check whether sea urchin core histones are capable of protecting the usual core-length DNA fragment, nucleosomes were reconstituted by reassociating long DNA by gradient dialysis with either total sea urchin sperm histone or total sperm core histones. These complexes were then digested with micrococcal nuclease. At short digestion times, broad bands of about 174, 334, 472 and 571 bp were observed for both types of reconstitute, corresponding to monomer, dimer, trimer and tetramer units. This implies that the higher multimers are close packed at a spacing of about 145 bp. With further digestion a monomer band is produced centred at 141 bp (see Fig. 4). These results indicate: (1) that in this type of reconstitute the H1 does not induce the native spacing of 241 bp and (2) that the core histones are able to protect a length of approx. 141 bp.

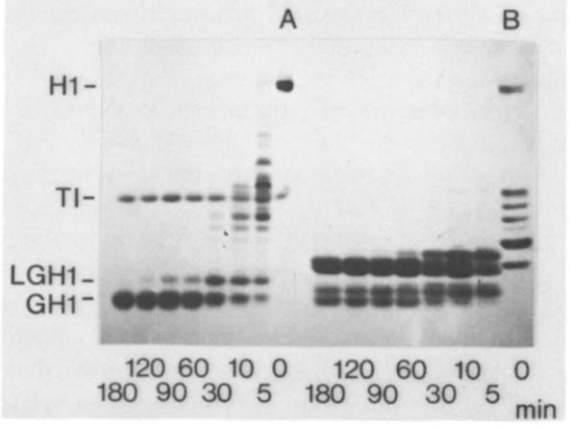

Fig. 3. SDS gel of protein products of *A. lixula* nuclei treated with trypsin for the times indicated. Panel A: proteins extracted with 5% perchloric acid and precipitated with trichloracetic acid, i.e., histone H1 and its products. Panel B: proteins precipitated by trichloracetic acid, i.e., total histone.

The 151 bp fragment seen in the chromatin digests falls midway between the observed core and chromatosome lengths in this tissue. Products of intermediate size are frequently seen in chromatin digests that retain H1 [30], where they are quite rapidly trimmed to 146 bp. In reconstitutes containing the H1 fragment NGH1 [33], they are also seen but are more resistant to trimming, rather as in the present case.

Chromatosomes

Since the 164 bp fragment was observed from the trypsin-treated chromatin in which the H1 had been degraded to the level of its globular domain (Fig. 2), it would seem that the globular domain alone is able to afford protection of the extra approx. 23 bp above the core particle length of 141 bp, as with other chromatin [18,31]. This conclusion was verified by reconstitution experiments in which H1/H5-depleted chicken erythrocyte chromatin was prepared in 80 mM NaCl. This chromatin material exhibits protection of only 146 bp core-particle-length DNA and various subnucleosomal bands [30,32]. Sperm histone H1s and their globular limit peptides (GH1s) prepared by trypsin digestion were reassociated by addition, also in 80 mM NaCl. Fig. 5 shows double-stranded DNA gels of the products of micrococcal nuclease digestion. Lanes A and B contain 1 and 4 min digests of the H1/H5-depleted chromatin reconstituted with intact H1 from *A. lixula* and *P. angulosus*, respectively, at a ratio (r) of 1 mol H1 per mol nucleosome. Lanes C give the chicken erythrocyte polynucleosome control. It is apparent that although a core-particle-length fragment of about 147 bp is well defined throughout, there is no clear evidence of protection of chromatosome-length DNA in either reconstitute. Neither is there any indication of the characteristic 151 bp band observed with nuclei.

Since prior treatment of sperm chromatin with trypsin led to a better definition of the 164 and 151 bp bands (Fig. 2a) as a result of removing both core histone tails and the N- and C-domains of H1, trypsin was also used with an *S. granularis* H1 reconstitute. Lanes E show a micrococcal nuclease digest of this reconstitute and lanes F the same treated with trypsin. After trypsinisation there is some indication of a chromatosome-length DNA, 168 bp. This result suggests that the globular domain of H1 (the product of H1 trypsinisation) is capable of chromatosome formation in a reconstitute. Reconstitution of just the globular domain of the H1 would thus be expected to show good 168 bp protection. This is seen to be so in lanes D in which just the GH1 fragment of *S. granularis* H1 has been reconstituted. Lanes D show the clearest protection of chromatosome length DNA that we have ever seen in this type of assay and show that the limit trypsin product of

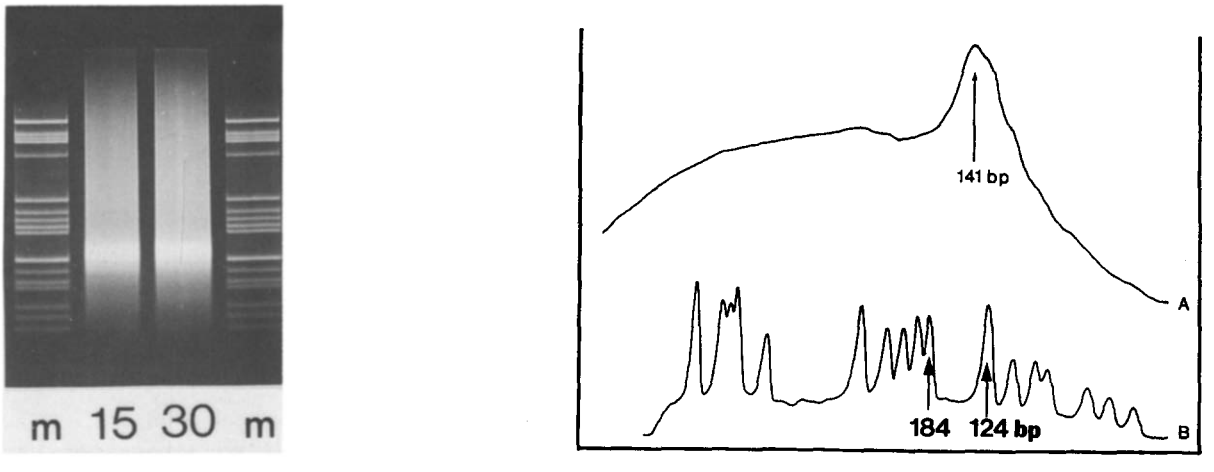

Fig. 4. Micrococcal nuclease digest of a reconstitute formed from total *A. lixula* sperm histone and long length DNA analysed on a 10% polyacrylamide double-stranded gel. Scan A shows the monomer length DNA after 30 min digestion. The marker, m, (scan B) is a *Hae*III/pBR322 digest.

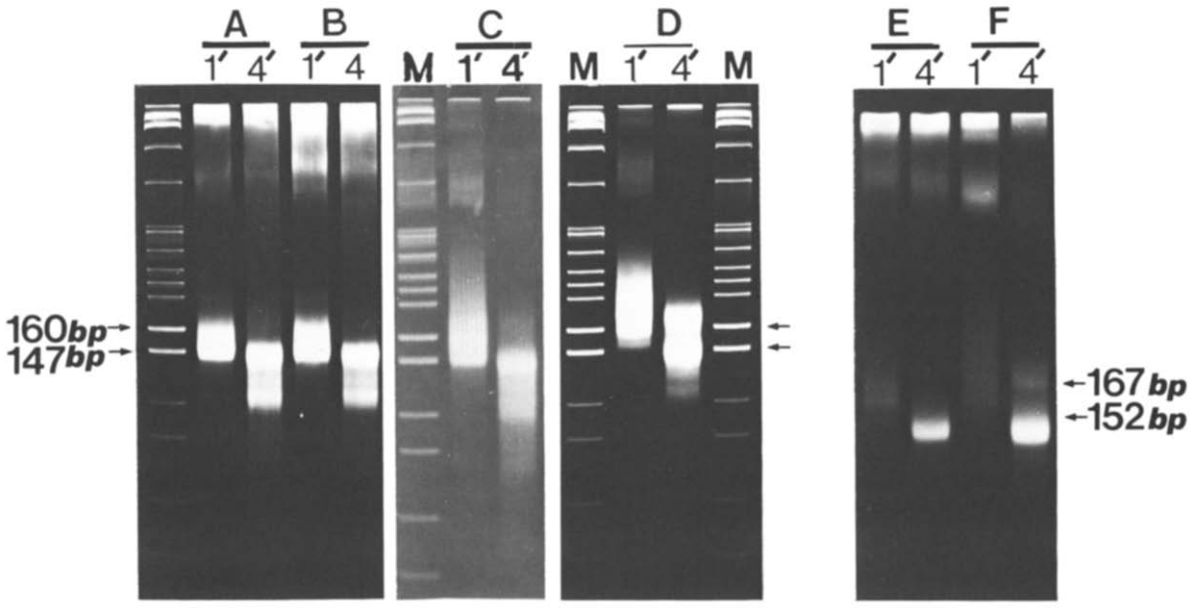

Fig. 5. Double-stranded DNA gels of the monomer products of micrococcal nuclease digestion of reconstitutes of H1/H5-depleted chicken erythrocyte polynucleosomes with sea urchin sperm H1 and its limit product GH1. All reconstitutes, at a molar ratio $r = 1$ of H1 (GH1) per nucleosome, treated with nuclease for 1 and 4 min. Marker M is *Hpa*II/pBR322 DNA and lanes E and F were referenced with respect to *Hae*III/PM2 DNA. Lanes A, intact *A. lixula* H1 reconstitute; lanes B, intact *P. angulosus* H1 reconstitute; lanes C, control of H1/H5 depleted chicken erythrocyte polynucleosomes; lanes D, *S. granularis* GH1 reconstitute; lanes E, intact *S. granularis* H1 reconstitute; lanes F, as lane E, but reconstitute treated with trypsin for 5 min before digestion with nuclease.

sea urchin H1 is able to form a chromatosome in the same way as the H1 from the more usual tissues [18,19]. It is therefore to be expected that in sea urchin sperm chromatin which has been trypsinised to the level of GH1, a chromatosome-type structure about 22 bp larger than the core particle is observed.

Why, then, is intact H1 unable to protect chromatosome-length DNA in the reconstitute? One explanation is that the very high arginine content results in very tight binding to DNA (0.75 M NaCl is required to release the H1 from chromatin [34]). As a consequence there is little redistribution of the H1 following its random initial binding to the chicken erythrocyte polynucleosomes in 90 mM NaCl and it cannot find its correct binding site.

The globular domain of sperm H1

Although the complete sequence of H1 from one sea urchin sperm has been determined (*P. angulosus* [2]), and partial sequences from several others [3,4], the location of the tryptic globular domain within them has not been defined. The globular domain from *A. lixula* H1 was therefore prepared by free solution digestion with trypsin, as in Ref. 24, since it was shown there that the product of such a digestion is the same as that from chromatin. The free solution peptide is the same as that labelled GH1 in Fig. 3. This peptide was sequenced by automated Edman degradation for 49 cycles. The yields of amino acids at each stage are tabulated in the supplementary material (see footnote concerning BBA Data Deposition at the beginning of this article). For the first five cycles two residues were released as follows. Cycle 1, Ala/Arg; cycle 2, Lys/Ala; cycle 3, Pro/Lys; cycle 4, Ala/Pro; cycle 5, Ser/Ala. Thereafter, a distinct single sequence was observed. It is clear that two sequences are present displaced by one residue: the major component starts with Ala and continues with Lys-Pro-Ala-Ser, whereas the minor starts with Arg and is followed by the Ala of the first sequence. The complete results are given in

Fig. 6, which compares the sequence obtained with the corresponding region of *P. angulosus* sperm H1, calf thymus H1 (subfraction CTL1) and chicken erythrocyte H5. *A. lixula* GH1 has been given a total of 83 residues, since this was indicated by the amino-acid composition of the sequenced fragment (not shown). This corresponded closely to the composition determined previously and confirms the presence of two histidines, two tyrosines and one phenylalanine in the fragment [24]. The *A. lixula* sequence differs slightly from the corresponding region of *P. angulosus,* but the majority of the variations can in fact be found in the partial sequences of *E. crassa* or *S. granularis* sperm H1s [3,4]. The N-terminal point of *A. lixula* GH1 corresponds closely to those of calf GH1 and chicken GH5. Since the length of all three peptides is closely similar, it follows that the C-terminal points are at closely corresponding positions in the sequences. The tryptic product of sea urchin sperm H1s is thus completely analogous to those of other members of the H1 family.

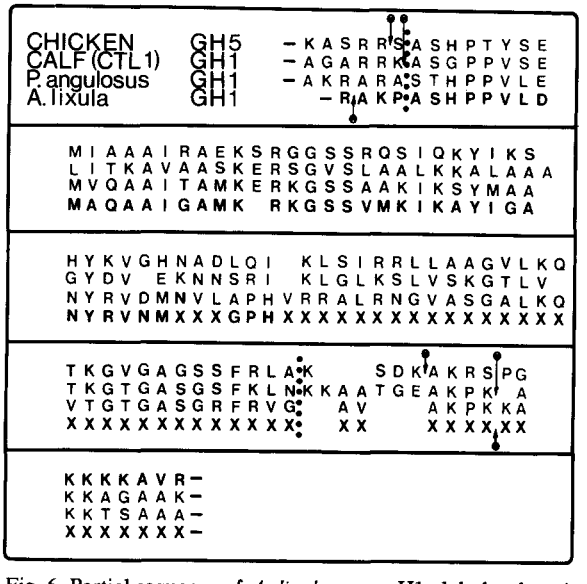

Fig. 6. Partial sequence of *A. lixula* sperm H1 globular domain (GH1) compared with those of calf thymus GH1 [38] and chicken erythrocyte GH5 [7]. The corresponding region of the *P. angulosus* sperm H1 sequence is also included [2]. Arrows indicate tryptic cutting points and dotted lines the limits of the conserved sequence region.

Sperm specific H1s

H1 species from sea urchin sperm are typically longer than those from calf thymus; e.g., that of *P. angulosus* contains 248 residues [2], *E. crassa* 252 residues [3] and *S. granularis* 250 residues [4]. These sea urchin sperm H1s have been reported to exhibit a special structural feature not seen with calf H1 or chicken H5. The total helicity of *S. granularis* H1 has been estimated as about 27%, i.e., about 64 residues, whereas that of calf H1 is about 13%, i.e., about 28 residues [23]. When the helicity of calf and sea urchin H1 limit peptides (GH1s) was compared, it was found that calf GH1 contained all the helical residues of the intact parent protein, but GH1 from *S. granularis* H1 had lost about half of the helical residues of the original molecule, leaving about 28 residues. This conclusion was reached on the basis of ellipticity measurements at 222 nm made on a single sea urchin H1 species [23]. We therefore recorded CD spectra of sea urchin sperm H1s from three sea urchins: *A. lixula, S. granularis* and *P. lividus.* Table I shows ellipticities recorded at two wavelengths sensitive to the amount of helix: 222 and 187 nm. It is immediately apparent that the ellipticities of 187 nm are in accord with those at 222 nm in indicating an approx. 2-fold greater helicity for the sea urchin sperm H1 molecules relative to calf H1 and chicken H5. There is no doubt, therefore, that all sea urchin sperm H1s contain considerably more helix than do somatic H1s, chicken H5 or bovine H1° [35].

It was previously shown [23] that on trypsin digestion of the H1 from *S. granularis* sperm to generate the limit fragment GH1, there is a rapid loss of the extra helix in the first phase of digestion (about 36 residues lost, corresponding to a drop in $-[\theta]_{222}$ from about 8000° to about 4000°), and this is followed by a very gradual loss of the remaining 28 residues, corresponding to slow degradation of the GH1 fragment. This experiment has also been carried out with *A. lixula* sperm H1 (see Fig. 7a) and a very similar result has been obtained. In contrast, calf H1 shows no rapid loss of helix, only the slow degradation of the globular domain fragment. We therefore conclude that the rapid loss of about 36 helical residues is a feature of all sea urchin sperm H1s and corresponds to a readily accessible region of the

TABLE I

ELLIPTICITIES OF THREE SEA URCHIN SPERM H1s AND TWO OTHER H1 FAMILY MOLECULES MEASURED AT TWO WAVELENGTHS RESPONSIVE TO THE CONTENT OF HELIX

Units of deg·cm^2·dmol^{-1}. The amount of helix has been estimated from the values at 222 nm assuming the absence of beta structure, a fully helical ellipticity of −30000° [36] and a random coil ellipticity of −1000° [37].

	$[\theta]_{222}$	$[\theta]_{190}$	Helix$_{222}$
A. lixula H1	−7500°	+9500°	26%
S. granularis H1	−8800°	+12600°	30%
P. lividus H1	−8000°	+10000°	28%
Calf thymus H1	−4700°	+6200°	16%
Chicken H5	−4300°	+4200°	15%

protein that nevertheless contains secondary structure.

Bearing in mind the strong sequence homology between the globular domain fragments (GH1s) of sea urchin sperm H1 and the other H1-family molecules (Fig. 6), their identity of helix content is to be expected. The extra helix in the sperm H1s is therefore located in what are usually considered as the flanking N- and C-domains. These do not, however, have a sequence that would seem able to support helical structure. The N-domain is very basic and contains much proline and serine, whilst the C-domain is of similar composition but includes a greater proportion of alanine at the expense of serine. Despite the apparent unsuitability of sequence, there must be helix in the sperm H1s outside the globular domain. Could this helix be remote from the globular domain, e.g., towards the end of C-domain? Fig. 7b shows the formation of secondary structure induced by ionic strength increase. For both H1 samples, but particularly for *S. granularis*, there is no sign of a two-step process that would indicate the existence of structurally separated folded region; it appears, rather, that all the secondary structure is in a single cooperatively folding domain. This suggests that the additional helix in sea urchin H1s is located close to the globular fragment. If present as a single region, these approx. 35 extra helical residues must be C-terminal, i.e., run between residues 120 and 155. Indeed, there is no proline in this region of the sequenced H1s from the sea

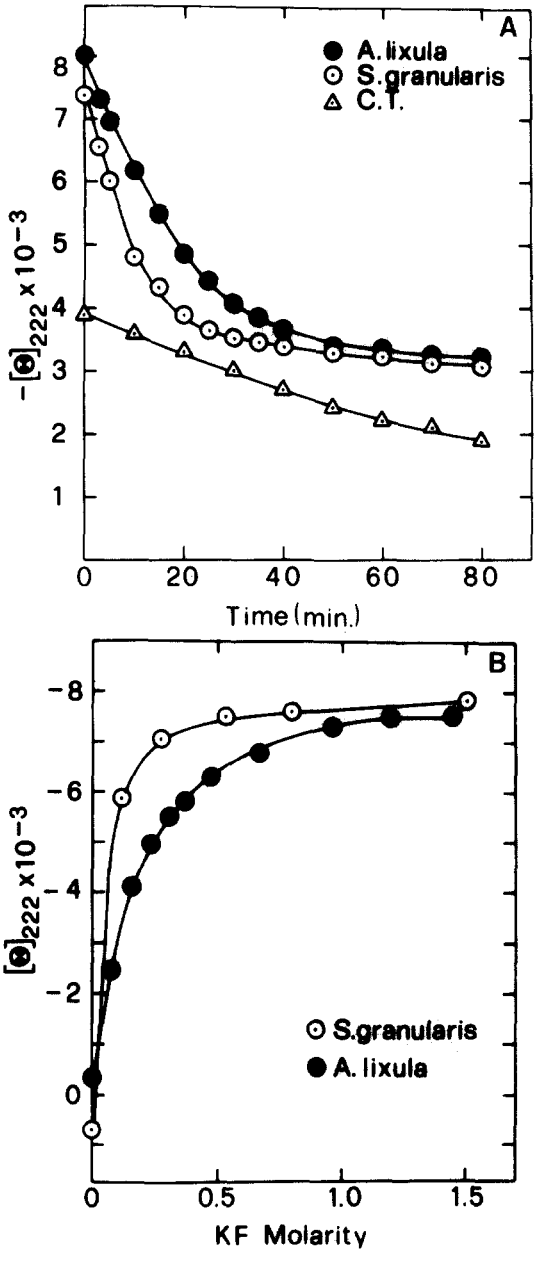

Fig. 7. (A) Time-course of trypsin digestion of H1 species in free solution in 1.5 M NaCl/20 mM Tris-HCl (pH 8) monitored at 222 nm. CT, calf thymus. (B) Salt dependence of secondary structure formation in *S. granularis* and *A. lixula* H1 molecules in free solution at pH 8, monitored at 222 nm.

urchins *P. angulosus* and *E. crassa*. An alternative would be that some (about 10) of the 'extra' helical residues were N-terminal to the globular

domain, e.g., from residues 31 to 40 of *P. angulosus* (there are several prolines before residue 31), whilst the remaining extra helical residues were C-terminal to the globular domain. These are regions of significantly lower sequence conservation than the globular domain of the sea urchin H1 sequences. In the perchloric acid extract of the tryptic digest of the sea urchin chromatin (Fig. 3) there is a prominent fragment running somewhat more slowly than GH1. This peptide is also seen in free solution digests and has been designated LGH1 [24]. It is, however, only about 12 residues longer than GH1 and so is too short to be a protected fragment containing all the extra helix of the intact protein.

Conclusions

It is shown that sea urchin sperm core histones are able to protect a core length DNA fragment of about 141 bp from micrococcal nuclease digestion. Likewise, a tryptic limit fragment of the sperm H1 molecule, consisting of about 83 residues and homologous to the corresponding fragments of calf H1 and chicken H5, is able to protect a fragment about 22 bp longer than the core length, i.e., about 163 bp. Since a 164 bp fragment is protected in the chromatin, these results are taken to indicate the presence of a chromatosome-type structure. Both core and chromatosome lengths appear to be about 4 bp less than in other chromatins and this might therefore mean that the DNA in sea urchin sperm chromatin is more tightly wound around the histones. However, the spread of fragment sizes observed for both core and chromatosome is too great to be certain of such a difference. The resistant fragment of 151 bp in this chromatin is, however, unusual, as is the inability of the nuclease to trim back to core particle size. Since the 151 bp length is about midway between the core and chromatosome length observed in this system, we conclude that it results from asymmetric endonucleolytic action on the chromatosome that is not matched by a corresponding symmetric cut. As a result no core particle is produced. This must be caused by the very condensed nature of the chromatin, which is not relieved by trypsin treatment, probably as a result of the high arginine content of the H1 histone, i.e., of its fragments which do not disperse from the chromatin but remain bound.

Despite these detailed differences from the more usual chicken erythrocyte or calf thymus chromatin, the observations reported here indicate that sea urchin sperm chromatin is constructed from the same general features of core and chromatosome, but modulated by the specific tight binding properties of its histones, particularly H1, that give rise to a very much more compact structure.

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