Chromatin from sperm of Bivalvia molluscs

Specific features of nucleosomal organization

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In chromatin from the sperm of two Bivalvia molluscs a highly basic low molecular mass sperm-specific protein (S-protein) has been found in addition to the full complement of histones. It is shown that sperm chromatin preserves nucleosomal organization, some parameters of which are specific: (i) DNase I cuts DNA within the sperm nuclei with dinucleosomal periodicity; (ii) linker DNA of the nucleosome is significantly elongated. The S-protein is shown to be located on the linker DNA.

Chromatin structure *Nucleosomal organization* *Nucleosomal linker DNA* *Sperm-specific chromosomal protein*

1. INTRODUCTION

During spermatogenesis alterations in composition of the basic chromosomal proteins take place in the majority of animals [1]. It has been suggested that the sperm-specific proteins control condensation of the sperm chromatin [1]. In this work changes in the basic chromosomal proteins are analysed in connection with specific features of compact sperm chromatin.

Earlier we showed that in sperm of the Bivalvia molluscs *Swiftopecten swifti* and *Glycymeris yessoensis* all histone fractions were present; the histone H1, however, was replaced by its sperm-specific variant enriched in arginine [2–4]. Besides, the highly basic sperm-specific low molecular mass protein (S-protein) is found in both molluscs in addition to the histones [2–4]. Here we report that the sperm chromatin from both species preserves a nucleosomal organization, some parameters of which are, however, specific: they include (i) dinucleosomal periodicity as revealed by DNase I and (ii) a significant elongation of the linker part of the nucleosome. The sperm-specific basic chromosomal proteins are shown to be located on the linker DNA.

2. MATERIALS AND METHODS

Sperm cells from the Bivalvia molluscs *S. swifti* and *G. yessoensis* (Bay of Peter the Great, Sea of Japan) were used. Collection of sperm, preparation of nuclei and isolation of the basic chromosomal proteins have been described [5]. Electrophoresis of proteins was according to [6,7]. Digestion of the nuclei with DNase I and micrococcal nuclease, preparation of nucleosomes and their separation on 5% polyacrylamide gel were as in [8]. Electrophoresis of chromosomal proteins in the second dimension was performed in an acetic acid-urea system in the presence of cetyltrimethylammonium bromide (CTAB) as in [9], except that the temperature was 40°C and CTAB was not present in the gel. To estimate the length of DNA in mononucleosomal subfractions the corresponding bands were excised from the 5% gel, incubated in the presence of 8 M urea and 1% SDS for 5 min at 100°C, and placed on the top of the preelec-

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trophoresed 5–10% gradient polyacrylamide gel, containing 40 mM Tris-borate (pH 8.3), 1 mM EDTA, 0.1% SDS and 8 M urea.

DNA isolation, agarose gel electrophoresis and determination of the DNA repeat length were as in [8].

3. RESULTS AND DISCUSSION

The sperm chromatin structure of the Bivalvia molluscs *S. swifti* and *G. yessoensis* was analysed using micrococcal nuclease and DNase I. Fig.1 presents agarose gel electrophoresis of double-stranded DNA fragments from the molluscs' sperm nuclei digested by micrococcal nuclease. The data clearly show that the sperm chromatin preserves periodic organization, the value of the DNA repeat length, however, is significantly greater (226 ± 3 bp in *Swiftopecten* and 223 ± 4 bp in *Glycymeris*) than that of somatic cells (table 1). The high DNA repeat value is due to elongation of the linker DNA since the length of core DNA is invariable (see fig.4). An increase in nucleosomal DNA repeat length has been also reported for avian erythrocytes and sperm from sea urchin, starfish and Holothuria [8,10–12]. Thus, it seems probable that elongation of the linker DNA in a nucleosome is characteristic of compact chromatin.

Another peculiarity that has been found both for avian erythrocytes and sea urchin sperm chromatin is a dinucleosomal periodicity of the DNA fragmentation resulting from DNase I treatment of the nuclei and displayed on agarose gel

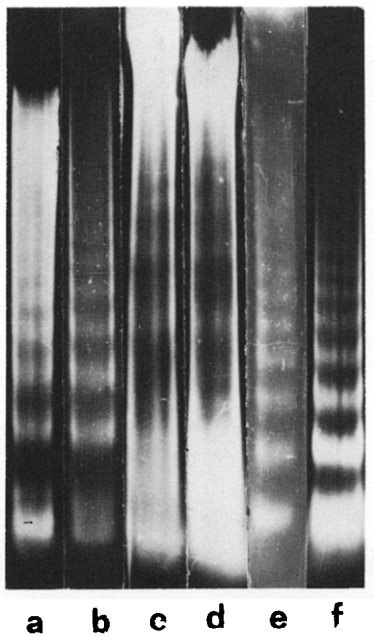

Fig.1. Agarose gel electrophoresis of DNA fragments from micrococcal (a,b,e,f) and DNase I (c,d) digests of nucleic from sperm of *S. swifti* (a,c) and of *G. yessoensis* (b,d). DNA fragments from liver nuclei of *S. swifti* (e) and rat (f) are given as standards.

under nondenaturing conditions [11,13]. This specific cutting of DNA with DNase I is also observed in sperm chromatin of both molluscs studied (fig.1c,d). The structural basis for the dinucleosomal periodicity of compact chromatin from erythrocyte and sperm cells remains un-

Table 1

Sizes of DNA fragments isolated from micrococcal digests of rat liver and Bivalvia mollusc liver and sperm nuclei, determined as in [8]

Band number	Base pairs				
	rat liver marker-liver	*S. swifti sis* liver	*G. yessoen-* sperm	*S. Swifti sis* sperm	*G. yessoen-*
2	370	370	355	450	445
3	570	572	555	683	670
4	775	775	760	900	900
5	960	965	920	1125	1112
6	1150	1150			
7	1350	1358			
Slope	195 ± 3	196 ± 3	191 ± 6	226 ± 3	223 ± 4

Data calculated as average of 5–7 electrophoreses

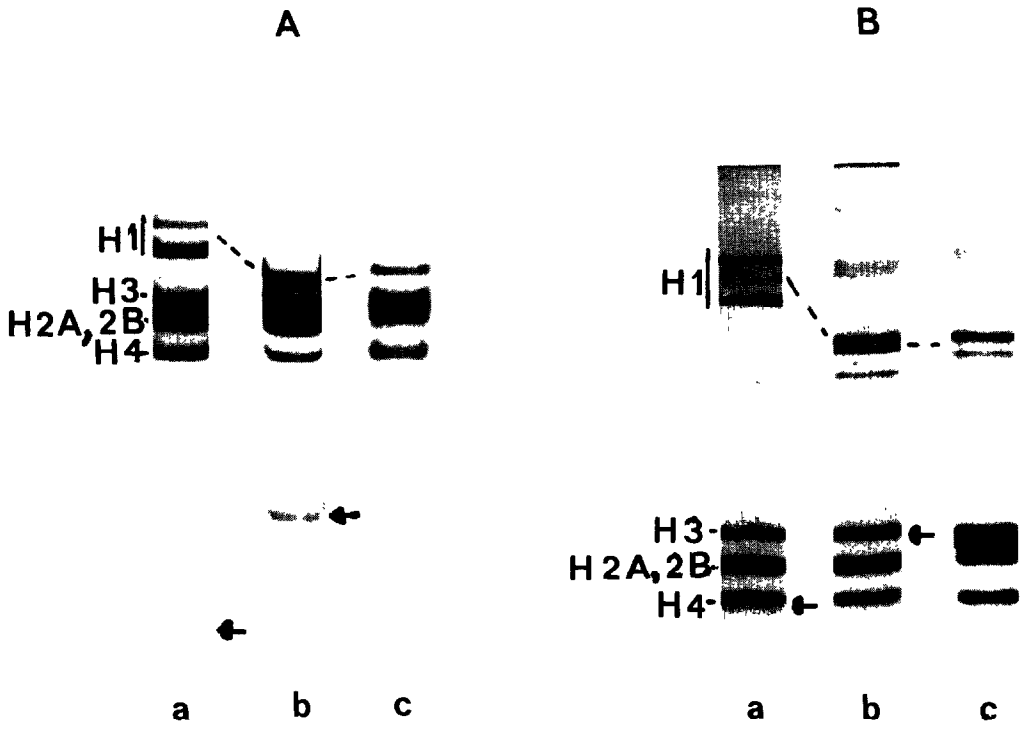

Fig.2. Polyacrylamide gel electrophoresis of the basic chromosomal proteins from the sperm of the Bivalvia molluscs (a) *S. swifti,* (b) *G. yessoensis* and from rat thymus (c). (A) According to [6]. (B) According to [7]. The position of the S-protein is indicated by the arrow.

known. A suggestion has been made that it may reside in unique H1 or H5 histones [11,13].

In the sperm of the molluscs all histone fractions are present (fig.2). Histone H1 in both species is represented by a sperm-specific variant enriched in arginine (8–11 mol%) [2–4]. Besides the histones, the sperm chromatin contains a highly basic low molecular mass sperm-specific protein (S-protein) [2–4]. The S-proteins have a mobility in acetic acid-urea gel electrophoresis much higher than that of the histones (fig.2A). In the presence of SDS (fig.2B) they migrate in the region of the histones (data of two-dimensional electrophoresis [4]) which makes their detection in an SDS system difficult. The S-proteins from *Swiftopecten* and *Glycymeris* differ in electrophoretic mobility (fig.2A). As determined by the method of incomplete succinylation [14] the S-protein from *Swiftopecten* contains 54 and that from *Glycymeris* 111 amino acid residues [15], more than half of them being basic. The S-proteins were extracted together with histone H1 from the chromatin or from the total basic protein with 0.5 N $HClO_4$.

Similar proteins were found in the sperm of other molluscs, some echinoderms and worms [1–5, 12, 16–23]. They are all characterized by a high content of basic amino acids (30–60 mol%), the lysine content being 20–50% mol% [2–4, 12, 15–22]. Different authors call them sperm histones, protamine-like proteins, etc. By extractability and amino acid composition S-proteins show some similarity to histone H1. The high positive charge density of the S-proteins and of the sperm histone H1 (0.5–0.6 and 0.33–0.37 positive charge per amino acid residue, respectively, [15]) obviously makes their interaction with DNA especially strong, which may underlie the mechanism of chromatin condensation.

The location of the sperm-specific proteins in the chromatin was determined using two-dimensional electrophoresis of nucleosomes (fig.3).

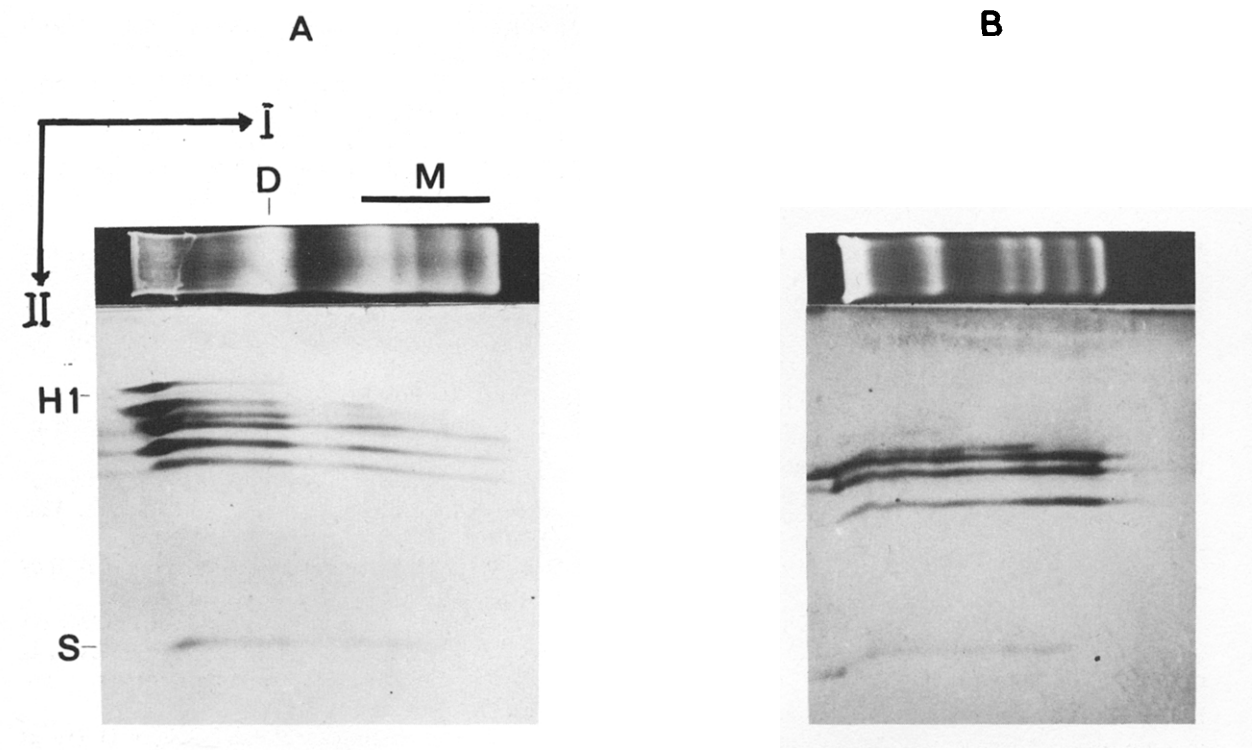

Fig.3. Two-dimensional electrophoresis of the nucleosome from the sperm of the molluscs (A) *S. swifti* and (B) *G. yessoensis*. (I) First dimension - low ionic strength electrophoresis of chromatin fragments on 5% polyacrylamide gel; (II) second dimension - separation of the nucleosomal proteins on 15% polyacrylamide gel in an acetic acid-urea-CTAB system. M, mononucleosomes; D, dinucleosomes.

First-dimension low ionic strength electrophoresis of the chromatin fragments released after micrococcal digestion of the sperm nuclei shows that the mononucleosomal zone consists of at least 4 subfractions. Analysis of the protein content of the nucleosomes by electrophoresis in the second dimension was performed using an acetic acid-urea-CTAB system (since in SDS gels the S-proteins comigrate with the core histones). Oligo-, tri- and dinucleosomes, as well as the 2 mononucleosomal subfractions with the lowest mobility contain a full complement of the chromosomal proteins: core histones, histone H1 and S-protein. The mononucleosomal subfraction with the highest mobility corresponds to the core particle: only core histones are present. Of interest is a subfraction following the core particle in which, again, histone H1 is absent. At the same time this mononucleosome contains, alongside the core histones, the S-protein. Thus, the S-protein is released from the nucleosome when the linker part is cleaved or

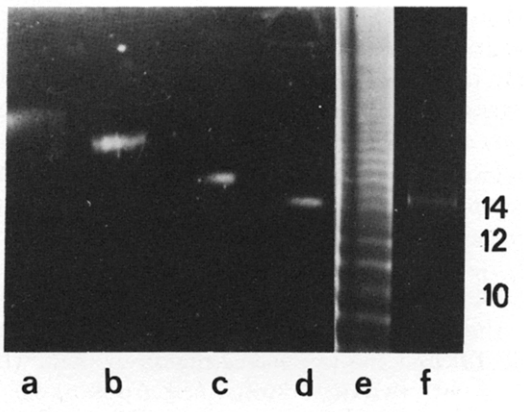

Fig.4. DNA from the mononucleosomal subfractions of *S. swifti* sperm nuclei in the order of mobility of the subfractions, from lowest to fastest (a–d); (d) core particle. DNase I digest of rat liver nuclei (e); DNA isolated from core particle of rat liver (f). 5–10% polyacrylamide gel, denaturing conditions; band numbers were estimated as in [27].

degraded. The data demonstrate the similar location of histone H1 and S-protein: both interact with the linker DNA of a nucleosome. A similar pattern of mononucleosomal subfractions and their protein content was found in sperm chromatin of Holothuria [23].

The length of DNA from the 4 mononucleosomal subfractions was found to be 145, 165, 195 and 225 bp (fig.4). The DNA length of the particle that contains the S-protein and does not contain histone H1 is 165 bp. The DNA length of the smallest particle in which histone H1 is present is 195 bp. Therefore the 165 bp particle, containing histone H1 that has been reported for other cells and called a universal unit - chromatosome [24], is not found in the mollusc sperm chromatin. It is possible that in the sperm chromatin 2 turns of DNA might be stabilized around the histone core by S-protein.

Our preliminary data on the molar proportion of histone H1 and the S-protein have shown that 0.5 molecule of S protein plus 1.2–1.4 molecules of H1 in *Swiftopecten* and 0.7–0.8 molecule of S-protein plus 2.2 molecules of H1 in *Glycymeris* are present per nucleosome in sperm chromatin. The presence of more than one molecule of lysine-rich histone bound to a linker DNA has also been reported for erythrocyte chromatin.

A similar location of the S-protein and histone H1 in sperm chromatin as well as their other properties show that the S-protein may, like histone H1, be involved in chromatin condensation. We suggest that organization of the linker part of a nucleosome, which is dependent on the length of its DNA and the properties of the bound proteins, is important for chromatin higher order structure.

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