Protein Free Spreading Method for Electron Microscopic Studies of DNA Compaction Stimulated by Interactions with Histone H1 in Surface Films

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Abstract. The application of the protein-free spreading method for electron microscopic strudies of DNA-histone H1 interactions stimulating DNA compaction is described. The method is based on the formation of the thin film containing DNA molecules on the surface of the hypophase of histone H1 solution. Histone H1 molecules interact with the spread in the surface film DNA molecules giving rise to mainly intramolecular DNA-histone H1 complexes which are transferred to the electron microscopic grids and studied in EM. This method has many advantages over traditional EM methods of DNA-ligand interactions studies and gives new important information about DNA- histone H1 compaction. The protein free spreading technique was applied to studies of linear, circular DNA and complexes of kinetoplast DNA compaction stimulated by histone H1. The results show that histone H1 stimulates side-by-side association of DNA duplex fiber and the models of DNA folding in the compact structures observed are offered.

The protein free spreading technique can be applied to studies of D NA compaction with other types of DNA binding agents.

Introduction

We describe application of the protein free spreading method for electron microscopic strudies of DNA-histone H1 interactions stimulating DNA compaction. The method is based on the formation of the thin film containing individual DNA molecules on the surface of the hypophase of histone H1 solution. Histone H1 molecules interact with the spread in the surface film DNA molecules giving rise to intramolecular DNA-histone H1 complexes which are transferred to the electron miscroscopic grids and studied in EM. This method has many advantages over traditional EM methods of DNA-ligand interactions studies and gives new important information about DNA histone H1 compaction.

Investigations of principles of DNA packing into higher order structures are of importance as the structural changes involving folding and unfolding of certain portions of genome DNA are connected with regulation of genetic activity. DNA - histone H1 complexes as a useful model of DNA compaction *in vivo* were intensively studied by electron microscopy . But in most studies the DNA-histone H1 complexes structures visualized by EM had irregular organization and contained many DNA molecules. It is explained by the fact that DNA-histone H1 complexes were obtained by direct addition of histone H1 to DNA solution with subsequent formation of three-dimensional networks or large intermolecular aggregates. In such preparation the DNA–fiber segments cannot be traced for significant length and it is not possible to follow the sequence of DNA folding events.

On the contrary when DNA is contained in the surface film and interacts with histone H1 molecules penetrating from the hypophase preferably the intramolecular condensation of DNA is observed, stimulated by assosiation of duplex DNA-fibers in a “side-by-side” manner. Here we describe the results of studies

with the use of protein free spreading technique [1] of histone H1 stimulated compaction of linear and circular superhelical DNA preparations and also of kinetoplast DNA networks and separate circles.

The protein free method can be applied to formation and ultrastructural studies of DNA complexes with different ligands as well.

Principle and Description of Protein Free Film Method

Fig. 1 shows schematically the principle of the protein -free film method application to the studies of DNA-histone H1 (or other ligand) interaction.

The protein free spreading technique is based on the ability of the certain buffer solutions in the absence of any proteins (like traditionally used cytochrome c) to spread on the surface of the hypophase. The droplet of hyperphase was either directly applied on the hypophase or was slided on the surface of the bent glass slide. We commonly used 30-40 µl of 20 mM Tris-HCl or TEA-HCl buffer (pH 8,3) hyperphase containing 2-5 µg/ml DNA. The spreading was controlled by application of talk particles on the surface of hypophase. The DNA is picked up from the surface of the hypophase on the collodion or carbon film coated grids. The preparations were contrasted by Pt-Pd shadowing.

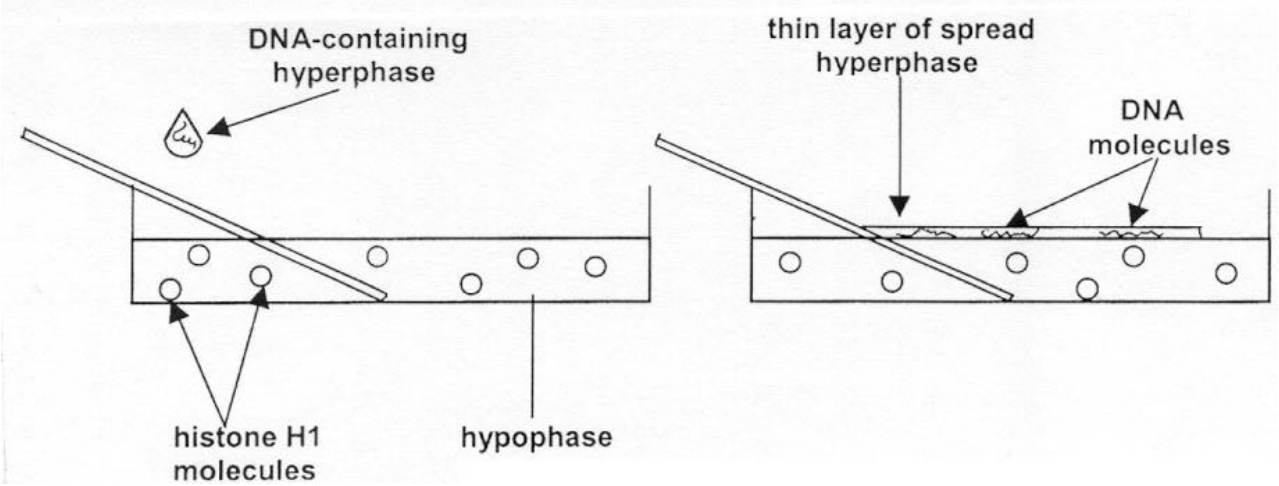


Fig. 1. The scheme of the protein-free film method

The EM analysis of the spreading of DNA on the hypophase without histone H1 shows that they are contained in the surface of the hypophase in a spread relaxed form as individual molecules.

If the ligand (in our case histone H1) is placed in the hypophase it penetrates the surface layer of the spread hyperphase and interacts with DNA molecules causing their compaction. At optimal DNA concentration it is possible to observe structures corresponding to individual DNA molecules compacted due to interaction with the ligand.

Compaction of Linear DNA.

Linear calf thymus DNA was spread on the surface of the hypophase in the absence and presence of different concentrations of histone H1. In the absence of histone H1 in the hypophase DNA molecules have the appearance characteristic of DNA spread by protein free method (Fig.2A). In the presence of 0.2-5 µg/ml of histone H1 in hypophase there are three types of structures on electronmicroscopic preparations: fibres of non compacted DNA (arrows in figs 2D,2E), compact fibres where on the periphery twisted strands of duplex DNA can be distinguished (Figs.2B, 2C) and compacted rod-like and circular structures where the separate fibres of duplex DNA are not seen (Figs.2D,2E). The study of compact structures morphology allows to conclude that they are formed by side-by-side association of DNA fibres, as it takes place in the formation of triple rings at the compaction of circular DNA with trivaline [2]. With the increase of ionic strength the tendency is observed for transition from second type structures to the more dense third

type structures. The latter can be explained by transition from non-cooperative to cooperative binding of histone H1 to DNA what probably stimulates more close spatial association of the DNA fibers.

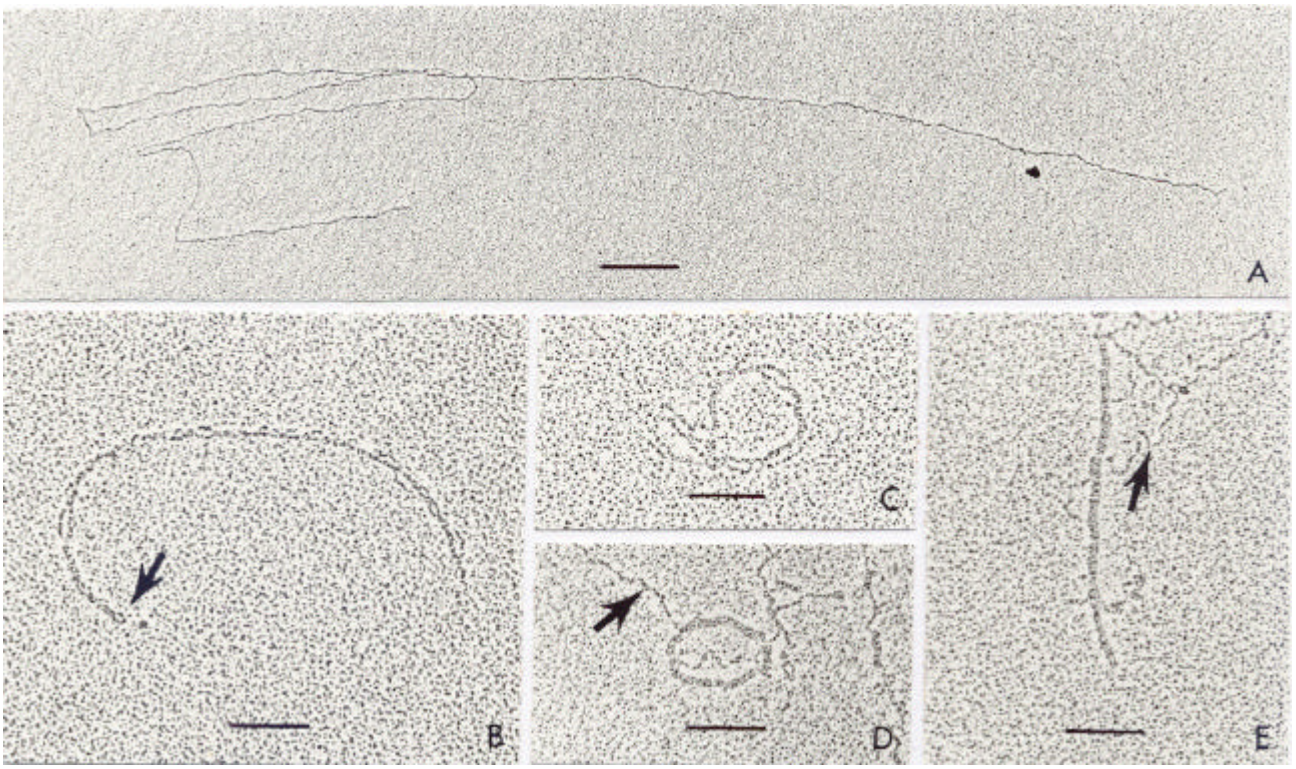
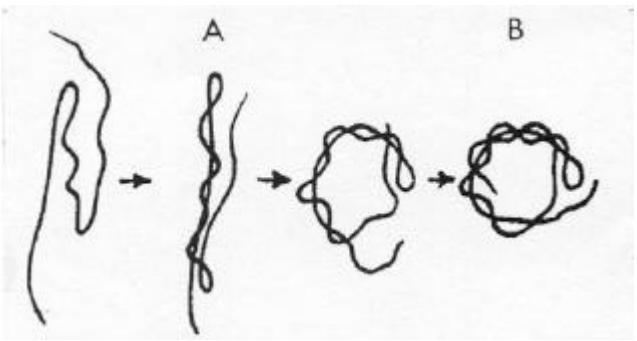


Fig.2. Calf thymus DNA, spread by protein free method on the hypophase without histone H1 (2A) and containing histone H1 with different concentrations (2B-E) . Bar indicates 0.2 mm.
(2 B,C) Rod-like (B) and ring-shaped (C) types of DNA-histone H1 complexes formed by the clearly seen twisted strands of duplex DNA. Arrow indicates the places where the fibre of duplex DNA make the 180 turn forming "a hairpin" .
(2D,E) Dense-structured DNA-histone H1 complexes. The separate segments of DNA fibres in ring – shaped (D) or rod-like (E) compact particles are not distinguishable. Arrows show the fibres of non-compacted DNA emerging from DNA-histone H1 complexes.

The described mechanisms of compaction behavior of DNA in the presence of histone H1 can be functioning in situations *in vivo* and the results obtained in the data can be of use for understanding of the role of histone H1 in higher order structure organization of chromatin [3].



The scheme in the fig. 3 shows the steps of DNA folding in the compact structures described.

Fig.3. The scheme of the linear DNA folding at different steps of rod-like (A) and ring-shaped (B) compact structures formation by DNA upon interactions with histone H1.

Compaction of Circular Superhelical DNA .

Circular superhelical DNA samples were spread on the surface of hypophase containing different concentrations of histone H1. In these conditions histone H1 molecules interact with individual DNA molecules contained in a thin surface layer of hyperphase and preferably intramolecular complexes are formed. Three DNA preparations (*p*Bl, *p*Lg19 and *p*Tbo1) having molecular sizes of 2920 b.p., 3820 b.p. and 6120 b.p. respectively were used. The conformational state of all DNA samples was checked by traditional *cyt C* method [4] and it was found that they have the conformation typical of circular superhelical molecules (figs. 4A, 4C, 4E).

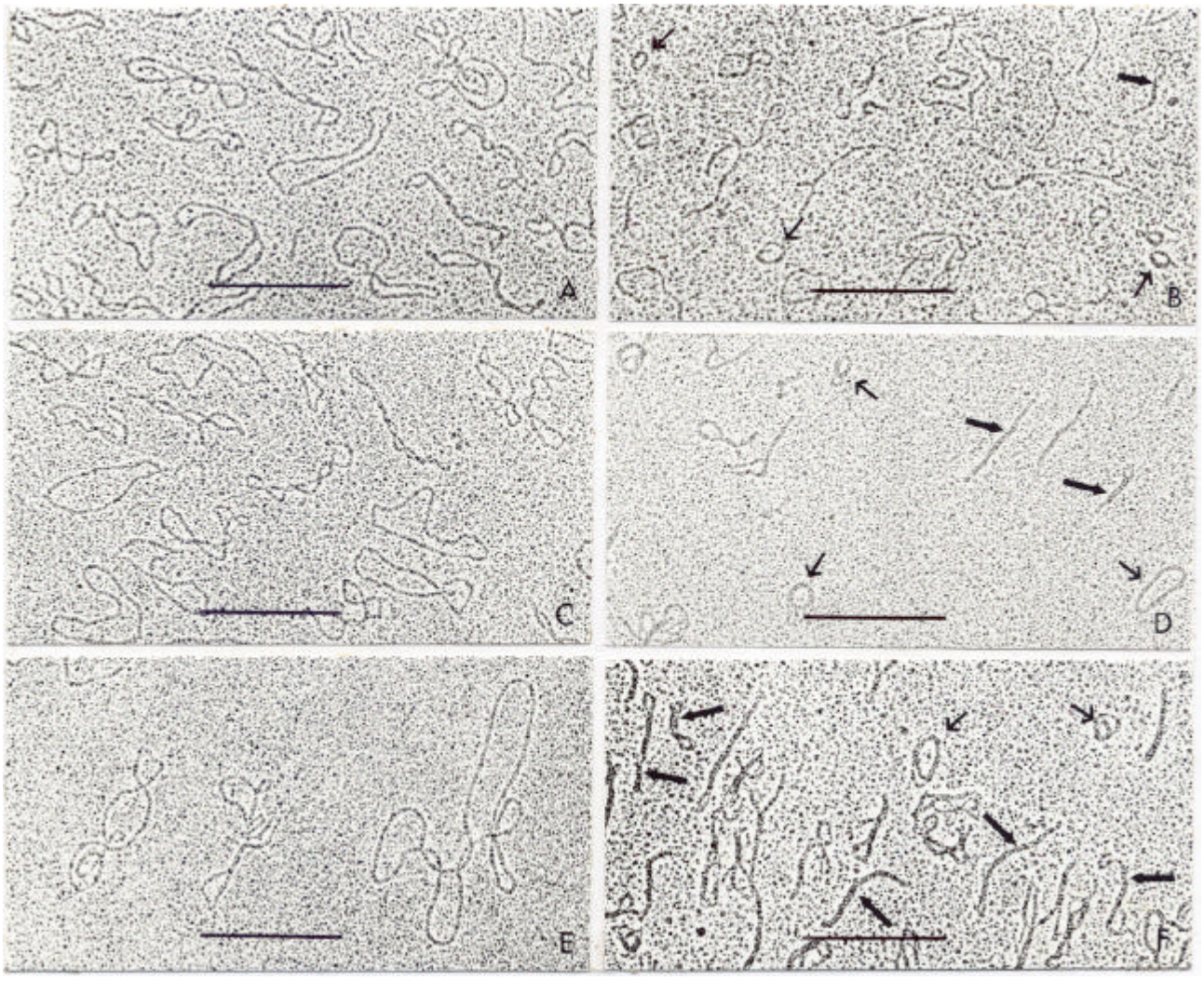


Fig. 4. Micrographs of protein film preparations of *p*Bl, *p*Lg19 and *p*Tbo1 plasmids (A,C,E), respectively and corresponding plasmid DNA-H1 complexes, prepared by protein free film technique (B,D,F) . Bar equals 0.5 μm.

On the micrographs of DNA-histone H1 complexes (figs. 4B, 4D, 4F) for all preparations along with certain amount of aggregates mostly individual compact particles of different morphology are present. Compact particles observed mainly have morphology of rods of varying thickness and length (indicated by thick arrows) and significant amount of circular compact particles (indicated by thin arrows). The rods often have small loops located at one or both ends of the structure (figs. 4B, 4D, 4F). The small loops protruding from fibers forming compact rings are also observed (figs 4B, 4D, 4F). According to earlier

results [3] and the morphology of the structures observed it is evident that compact structures are formed due to association of DNA duplex fiber of circular molecules in a side by side way.

Presence of fibers of different thickness forming both rods and rings allows to make an assumption that they relate to different compaction levels and probably contain different numbers of DNA duplex fibers arranged in a side by side way. The contour length of thicker rings is evidently much smaller than that of the thin rings. According to evaluation of the particle volumes on the base of the data of one side shadowing (fig. 5) most of the structures contain one DNA molecule. In general contour length measurements have shown considerable heterogeneity of the intramolecular compact particles of DNA-histone H1 complexes from the point of view of compaction level. It demonstrates that histone H1 is able to stimulate lateral association of DNA duplexes and of compact fibers of DNA-histone H1 complexes as well. As the longest rods evidently corresponding to the first level of molecule folding have the length equal to half of the contour length of the plasmids, contained in them it can be assumed that these rods are formed by aligned and attached to each other halves of the circular molecule. A model of ring particles formation can be proposed where rings are formed by overlapping and association of the ends of the rods (fig. 6). This model is similar to that proposed for linear DNA compaction (Fig. 3) and also explains the heterogeneity of the compact coefficient for ring structures as the different parts of rings contain different numbers of aligned DNA duplex fibers.

Fig. 5. One side shadowing of *p*Lg 19 DNA-H1 complexes. Bar equals 0.5 μm.

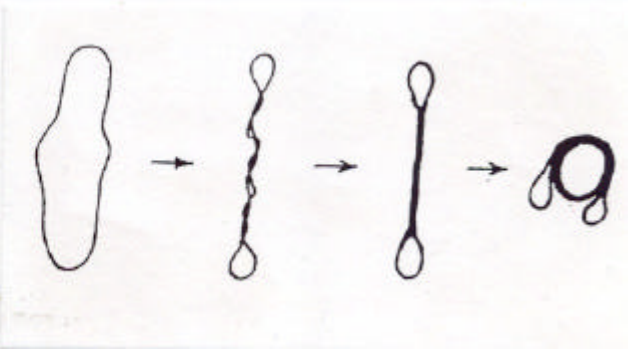


Fig. 6. Graphical presentation of a model of a probable pathway of compact ring structure formation.

Compaction of kpDNA

Kinetoplast DNA which is an association of DNA minicircles with bound maxicircle DNA molecules is contained in compact state which is provided by the bound basic histone-like proteins. We studied structural changes of isolated kpDNA caused by interaction with histone H1. KpDNA was spread on the surface of hypophase with different histone H1 concentrations with the use of protein free spreading method. The structural changes in both integral kpDNA networks and co-isolated separate minicircles were followed.

The micrographs of control preparation of kpDNA prepared with the use of protein technique by Davis [4] are shown in fig.6 A,B and have a typical appearance of a network of kinetoplast DNA. All the control preparations contain separate minicircle molecules on the periphery of the networks (arrows on Fig.6B). When histone H1 is present in the hypophase and the kpDNA is spread on the hypophase by protein free technique at the concentrations of 5-10 μg/ml of histone H1 in hypophase the networks have overall typical well-spread recognizable structure with the diameters similar to that of the networks in the control preparations. But the fibres from which the network is composed look more thick and are obviously formed by several dsDNA fibres lying side by side (Fig.6C).

Separate minicircles which are out of the contact with the network also change their appearance (Fig.6C, arrows). These changes are typical of the association of DNA segments in a side by side manner described by us earlier [2] for "triple rings". The tendency of minicircle compaction increases at the raise of histone H1 concentration in hypophase. Fig.7A shows the minicircles in the control preparations. The contour length of the molecules in these preparations is 0.88 ± 0.1 μm. Figs. 7B,C,D correspond to the concentrations of histone H1 1,5,10 μg/ml, respectively. At 1 μg/ml histone H1 concentration separate minicircles have the length 0.75 ±0.1 μm, at 5-10 μm/ml - 0.23 ±0.1 μm. This structural transition is accompanied by the thickening of fibres forming the compact circular structures. Obviously these fibres are formed by several DNA segments located in a side by side manner. The compaction coefficient for the observed structures is 3.7.

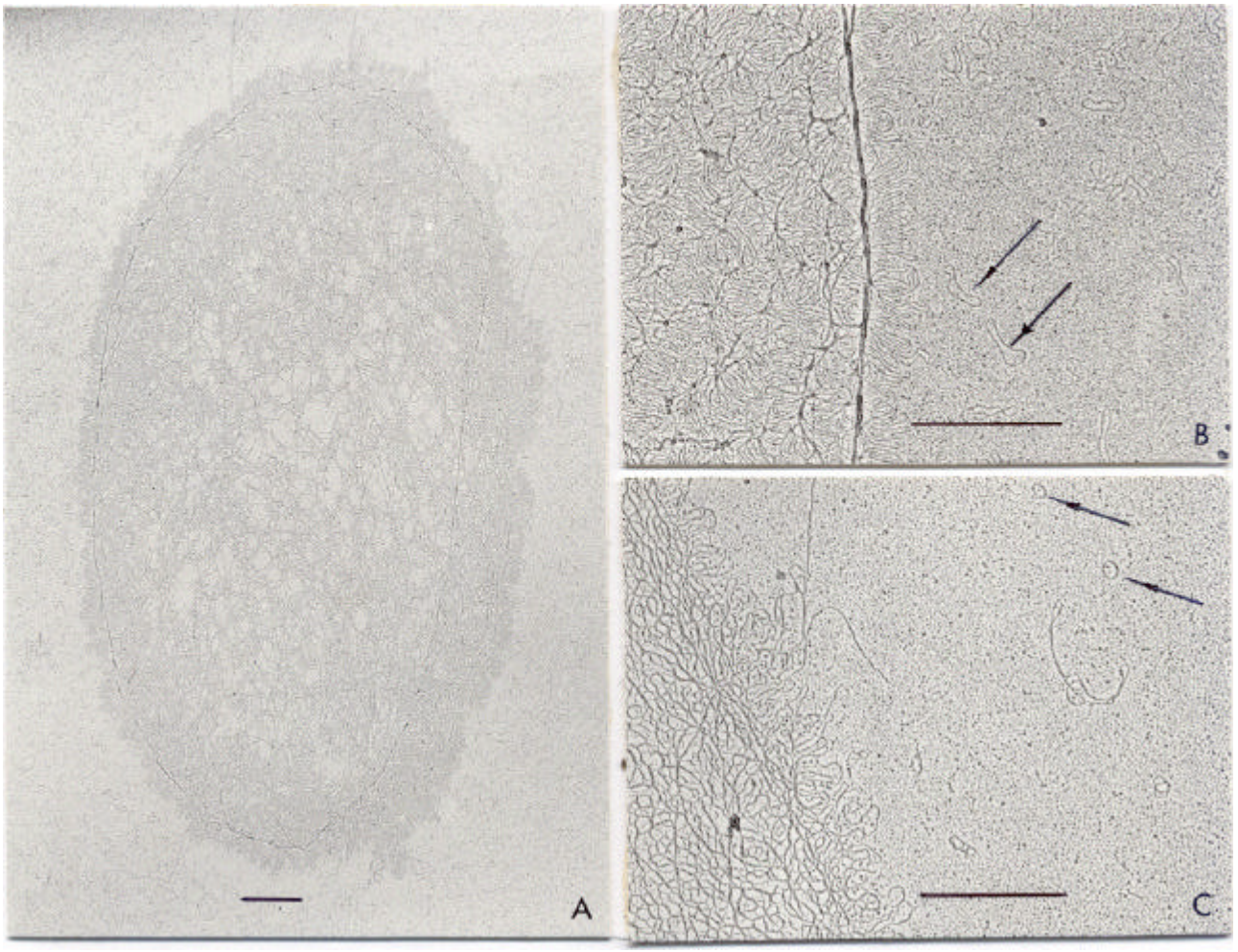


Fig. 6. (A) Control preparation of Crithidia fasciculata kinetoplast DNA, (B) Fragment of kpDNA network with minicircles (arrows), located in the proximity of network in the control preparations and (C) at 5 μg/ml histone H1 in hypophase. Bar equals 1 μm.

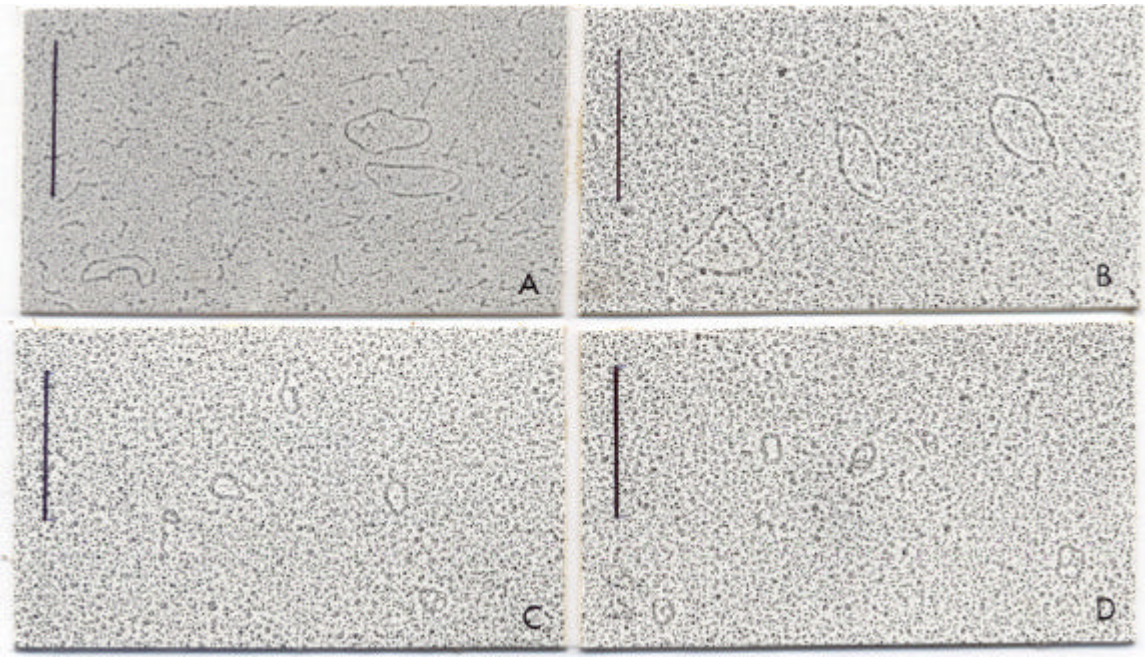


Fig. 7. Separate minicircles of kpDNA in control preparations (A) and at the concentrations of histone H1 – 1μg/ml (B); - 5 μg/ml (C); - 10 μg/ml (D). Bar equals 0.5μm.

Our data shows that histone H1 acts as an agent stimulating the side by side association of fibres within the networks of kinetoplast DNA without any linear compaction of the complete structure but simultaneously for separate short circular DNA molecules the same mechanism stimulates the compaction with the formation of compact circular particles [5].

Conclusions. The presented results demonstrate that the protein free spreading technique gives the possibility of the studies of different DNA preparations compaction upon interactions with histone H1 contained in hypophase. The spreading technique conserves the structure of isolated linear and circular DNA samples and of complicated DNA associates like kpDNA and allows to identify individual compacted molecules. This approach can be fruitful for the studies of DNA interactions with any kind of DNA binding ligands of different types.

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