Volume 322, number 3, 311–314 FEBS 12458 May 1993

Electron microscopic study of compaction of individual DNA molecules with histone H1 in surface films

Yuri Yu. Vengerov, Larissa P. Martinkina and Timur E. Semenov

V.A. Engelhardt Institute of Molecular Biology, The Russian Academy of Sciences, Vavilov str. 32, 117984 Moscow, Russian Federation

Received 22 March 1993

Interaction of individual spread DNA molecules with histone H1 was studied by electron microscopy using the protein-free spreading technique. It was shown that in the presence of 0.2–5 μg/ml of histone H1 in hypophase there are three types of structures in electron-microscopic preparations: fibres of non-compacted DNA, compact fibres with twisted strands of duplex DNA and compacted rod-like and circular structures where the separate fibres of duplex DNA could not be distinguished. The study of compact structure morphology allows us to conclude that they are formed by side-by-side association of DNA fibres. At an increase of ionic strength there is a tendency for transition from the second to the third type structures. The latter can be explained by transition from non-cooperative to cooperative binding of histone H1 to DNA. Regularities established for interaction between DNA and histone H1 can be useful for understanding the role of histone H1 in the higher order structure organization of chromatin.

DNA; Histone H1; Compaction; Protein-free film method

1. INTRODUCTION

Compact DNA structures arising as a result of interaction with different agents have been studied by many authors. These studies were aimed at elucidation of the main ways of DNA compact structure formation. Among a wide variety of different agents causing DNA condensation in vitro, one can find polyamines [1,2], polyethylene glycol [3], polyvalent cations [4] and others [5,6]. Similar experiments with histone H1 are particularly interesting due to its important role in packing the chromatin nucleosomal fibre into higher order structures in vivo [7].

In earlier studies it was shown that histone H1 upon interaction with DNA stimulated its condensation with formation of various structures: toroidal structures ('donuts') [8–10], large fibrillar aggregates [10,11], networks [12], and ring-shaped structures [11]. Physicochemical studies show that histone H1 binding to DNA depends on ionic strength, and an NaCl concentration increase within the range of 20–40 mM causes transition from non-cooperative to cooperative histone H1 binding [11].

But in most cited studies, the structures visualized by electron microscopy are not intramolecular and contain many DNA molecules. This is explained by the fact that H1–DNA complexes in these studies are formed by direct addition of H1 to DNA solution that causes an easy association of different DNA fibres, distributed in the vicinity of each other in the solution. The process gives rise to three-dimensional networks or large intermolecular aggregates in which significant portions of single DNA segments are inaccessible for studying the sequence of compaction events.

In our work we used the technique of DNA–histone complex preparation which allows us to obtain intramolecular compact structures, arising from interaction of initially spread individual DNA molecules with histone H1, penetrating from the hypophase to the surface layer. The results show that under these conditions histone H1 stimulates preferably the intramolecular association of duplex DNA fibres in a 'side-by-side' manner with formation of the rod-like and circular compact structures as it takes place in the case of the triple ring formation described for DNA complexes with a synthetic oligopeptide [13].

2. MATERIALS AND METHODS

Calf thymus DNA ('Sigma') was used. Histone H1 was prepared as described earlier [14].

The protein-free film technique [15] was used for DNA spreading on the hypophase, containing different concentrations of histone H1 in 0.14 M NaCl. For this purpose 30 μl of solution containing 5–10 μg/ml DNA in 30 mM TEA-HCl, pH 8.5, were overlayed on the hypophase consisting of histone H1 solution in a proper concentration placed in a tephlon tray. The spreading was controlled by talc particles loaded on the hypophase surface. DNA or DNA–histone H1 complexes were transferred to the freshly prepared and/or ion-charged grids, covered with the collodion supporting films. The preparations were air dried and rotary shadowed with Pt/Pd alloy (1:4). In some

Correspondence address: L.P. Martinkina, Laboratory of Electron Microscopy, Institute of Molecular Biology, Academy of Sciences of the USSR, Vavilov str. 32, Moscow, V-334, 117984, Russian Federation. Fax: (7) (095) 135 14 05.

cases the preparations were stained with 1% uranyl acetate before shadowing.

The preparations were analysed in a JEM-100CX electron microscope at 5,000–20,000 ×. The dimensions of structures were estimated on micrographs at the final magnificiation 25,000–50,000 ×.

3. RESULTS AND DISCUSSION

The DNA compaction upon its interaction with histone H1 was studied in experimental conditions providing the interaction of individual DNA molecules with histone H1 in the thin surface films. For this aim DNA was spread by the protein-free method [15] on the surface of a hypophase, containing histone H1 in the presence of different NaCl concentrations.

It is well known that DNA–histone H1 interaction causes DNA compaction. Histone H1 under described conditions condenses individual DNA molecules into the compact structures within a surface layer. We used electron microscopy to study the structures arising after DNA spreading on the hypophases containing various concentrations of histone H1 in bidistilled water or in the presence of 0.14 M NaCl.

The micrographs shown in Figs. 1–3 correspond to DNA (Fig. 1) and DNA–histone H1 complexes (Figs. 2 and 3) prepared as described. In the absence of histone H1 in the hypophase the DNA molecules (Fig. 1) have the appearance characteristic of DNA spread by the protein-free method [15].

It should be noted that when the preparations are made without histone H1 in the hypophase special care should be taken about the ability of the electron-microscopic support film to adhere DNA. It is recommended to use freshly prepared and/or ion-charged hydrophilic films to obtain reproducible results.

When histone H1 is added to the hypophase the morphology of the structures observed in the preparations changes drastically. On the one hand, the presence of histone H1 molecules in the hypophase considerably improves the material adsorption to the films. On the other hand, most structures observed in the preparation evidently are those corresponding to different stages of DNA compaction caused by its interaction with histone H1.

Different concentrations of histone H1 in the hypophase were used (from 0.2 to 5 μg/ml) and mainly three types of structures were observed in all preparations. The quantitative ratios of different morphology structures observed in the preparations depend on the ionic conditions and histone H1 concentration in the hypophase.

The first type corresponds to fibres of non-compacted DNA. These fibres look like DNA in control samples. They are present in all preparations and their relative content drops at higher histone H1 concentration in the hypophase.

The second type of structure is represented by compact fibres where the interwound DNA regions can be seen (Fig. 2). This type of morphology is characteristic of most of the structures observed when DNA is spread on the hypophase consisting of histone H1 solution in water.

It can be clearly seen from the micrographs in Fig. 2 that the rod-like and ring-shaped structures are not uniform in their thickness along their contour length and are formed by several side-by-side and interwound DNA duplex fibres. Despite the fact that several DNA fibres lying side-by-side can be distinguished in the fibres of condensed structures shown in Fig. 2 the number of single DNA molecule segments forming higher order structures cannot be determined. This number is most probably more than 3. Such an approximate evaluation is explained by the fact that in a compact fibre a single segment of double-stranded DNA cannot be visualized over a long distance.

Based on mean values of the molecular mass of DNA preparations and measurements of the contour length of ring and rod-like structures we conclude that most of these structures result from the intramolecular compaction of individual DNA molecules. The structures are observed in both the rings and rods where the DNA duplex fibre is seen to turn for 180° forming 'a hairpin'. Such hairpins are indicated by arrows on Fig.2a,d. Analysis of the rod and ring structures of the second type allows us to conclude that they are formed by the DNA fibres association in a 'side-by-side' manner. In their overall organization, these structures resemble 'triple rings' formed upon circular DNA interaction with a synthetic oligopeptide, trivaline [16].

The third type is represented by the dense compact rod-like or ring-shaped structures where individual duplex DNA fibres cannot be distinguished (Fig. 3). The thickness of such fibres on the rotary-shadowed preparations is 200–300 Å and they represent the major part of the structures in the DNA preparations spread on the hypophase of histone H1 in the presence of 0.14 M NaCl. In general, these compact structures do not have as regular organization as the second type structures. Very often, rather long segments of non-condensed DNA are observed in contact with these structures (arrows on Fig. 3a,c). Basically, the rings and rods of the third type are very similar in size and organization to those of the second type, but differ from them by the substructures of fibres, forming the corresponding structure.

Compact structures of the second type in which interwound duplex DNA fibres can be seen are more loose and structures of the third type are more condensed and are formed by more uniform fibres. The latter type of DNA–histone H1 complex according to its morphology corresponds to the fast sedimenting complex observed by Clark and Thomas [11]. The slower sedimenting DNA–histone H1 complex [11] is probably structurally similar to the second type structures (Fig. 2). This analogy is also confirmed by the fact that there is a tendency

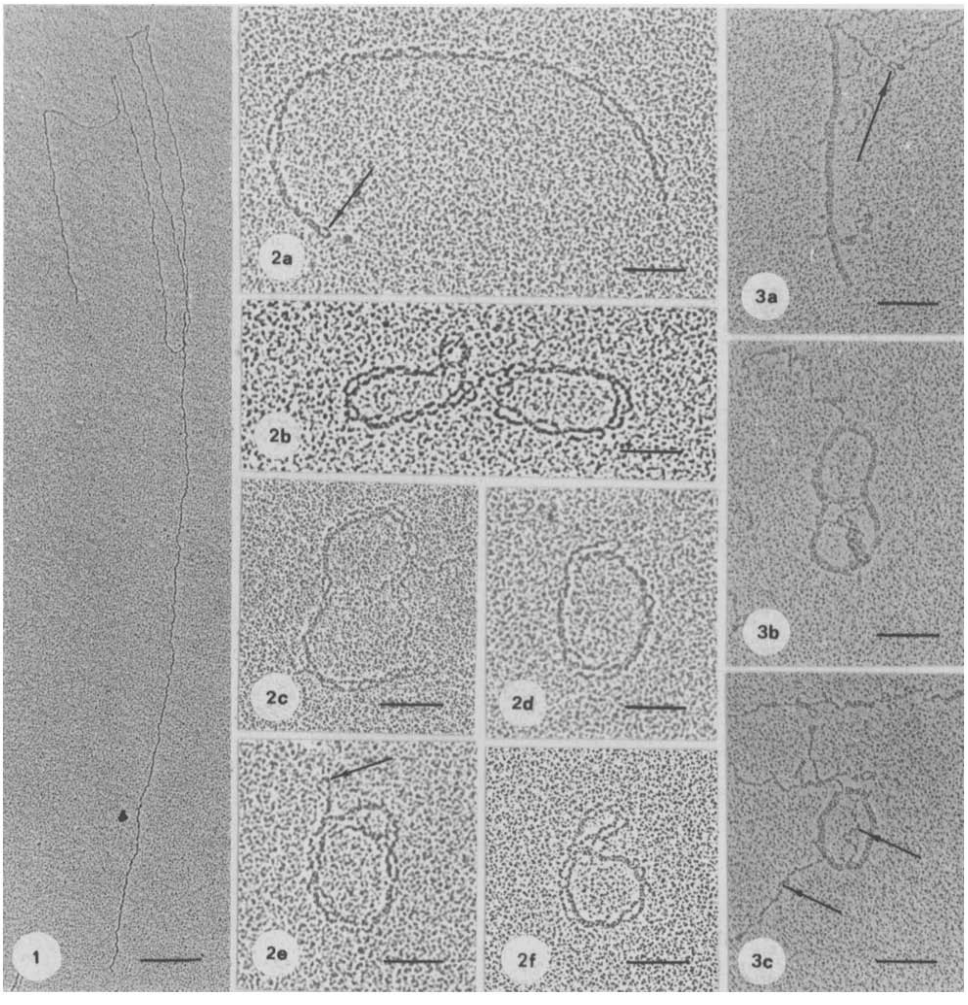

Fig. 1. Preparation of calf thymus DNA, isolated by the protein-free method. Bar = 0.2 μm.

Fig. 2a–f. DNA–histone H1 complexes (second type structures) formed on the bidistilled water hypophase. Arrows show the places where the fibres of duplex DNA make the 180° turn forming 'a hairpin'. Bar = 0.2 μm.

Fig. 3a–c. Third type of DNA–histone H1 complex formed on the 0.14 M NaCl hypophase. Arrows show the fibres of non-compacted DNA. Bar = 0.2 μm.

of transition from the second to the third type structures caused by an increase of NaCl concentration in hypophase. Clark and Thomas [11] explained the transition from the slow sedimenting complex to the fast sedimenting one by transition of non-cooperative histone–DNA binding to cooperative. The attentive analysis of organization of compact fibres forming rods and rings in the DNA–histone H1 complexes allows us to conclude that they are formed by segments of the same DNA molecule arranged in a 'side-by-side' way. The ring structures were observed also when other agents were used [2,6,11,16]. Probably the formation of rings in the DNA compaction process is a general characteristic of interactions with the agents stimulating DNA fibre association in the side-by-side manner.

As was noted by many authors, the interaction with several DNA fibres is characteristic of histone H1 and corresponds to its position in the points of 'entrance' and 'exit' of DNA in the nucleosome 'core-particle' [14,17]. Transition to the cooperative histone H1 binding causes considerable compaction of fibres but at the same time many non-condensed DNA segments appear in preparations. The presence of such non-condensed structures can be explained by H1 molecule redistribution which leaves certain DNA regions completely free or partially deprived of histone molecules. The differences in the overall appearance of the fibres in the type two and type three structures can be related to different content and arrangement of histone H1 molecules in the structures [13].

Acknowledgements: The authors are grateful to Dr. L.G. Nikolaev for preparations of histone H1.

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