A triple helix model for the structure of chromatin fiber

V. Makarov, S. Dimitrov+, V. Smirnov and I. Pashev+*

Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR, and +*Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

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A model of chromatin fiber structure is presented in which a repeating unit of a trinucleosome forms a 3-dimensional zigzag. Twisting and compression of the zigzag result in a triple helix structure. The model is built mainly on the flow linear dichroism data showing that (a) nucleosomal disc faces are tilted relative to the fiber axis, (b) the orientation of nucleosomes does not change upon folding and unfolding of chromatin, and (c) the orientation of nucleosomes is maintained by the globular domain of histone H1.

Chromatin structure *Flow linear dichroism* *Nucleosome orientation* *Histone H1*

1. INTRODUCTION

Two alternative types of higher order chromatin structure have been suggested so far: a regular helix or solenoid [1], and clusters of nucleosomes called 'superbeads' [2]. All specific models for packing of nucleosomes in chromatin fibers are based on the solenoid structure and represent either single regular helices [3–7] or double helical structures [8,9]. Uncertainty about the topology of DNA in chromatin does not allow a proper evaluation of the proposed models. Yet, based on their findings that nucleosomal discs are tilted from the fiber axis, Crothers et al. [10] claimed recently that models in which the discs are either parallel or perpendicular to the axis should be eliminated.

Here, we propose a triple helix model in which a repeating unit of a trinucleosome forms a 3-dimensional zigzag. The model is built on the flow linear dichroism measurements of chromatin preparations with different length of linker DNA, the studies on the role of histone H1 in maintaining the orientation of nucleosomes as well as on data reported from electron microscopy and other physical methods.

* To whom correspondence should be addressed

2. EXPERIMENTAL

2.1. *Flow linear dichroism of chromatin preparations with different length of linker DNA*

Recent studies employing flow linear dichroism (LD) showed that high molecular mass chromatin from rat liver and calf thymus (linker approx. 30 base pairs) exhibited a positive optical anisotropy in both the folded and unfolded state [11,12]. This means that the faces of nucleosomes are tilted from the fiber axis, the tilt being more than 36° (the 'magic angle' when the value of LD is zero). The average estimates of the tilt, assuming a supercoiled linker, were 36–39° [12,13]. Having in mind that when the angle of orientation of nucleosomes α is close to the magic angle the dichroism is sensitive to very small changes in α, the obtained quantitative changes in LD upon increasing the ionic strength from 2 to 100 mM NaCl [12] favored the conclusion that during the condensation of chromatin the orientation of the nucleosomal discs remained practically unchanged. This result was confirmed by our recent data showing no change in the sign of the dichroism upon trypsin-induced unfolding of chromatin [14].

To see whether such a conclusion is valid for chromatin from other species, as well as to

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estimate the contribution of linker DNA to the optical anisotropy of the chromatin fiber, we extended our LD studies to chromatin from Friend erythroleukemia cells, chicken erythrocytes and sea urchin sperm, where linker DNA is approx. 10, 45 and 80 base pairs, respectively. DNA in the different chromatin samples was of similar molecular mass, which allowed a quantitative comparison of the data obtained. The results presented in fig.1 clearly show that the difference in the linker length does not significantly affect the overall optical anisotropy of chromatin: in the salt range 2–100 mM all samples showed a positive dichroism with an amplitude similar to that observed with calf thymus chromatin. The finding that the orientation of nucleosomes is preserved upon folding and unfolding of chromatin was the starting point for the proposed model of the chromatin fiber.

2.2. *Flow linear dichroism of trypsinized chromatin*

The role of histones and their different domains in the maintenance of the orientation of nucleosomes was investigated using trypsin-cleaved chromatin. It was found that the positive dichroism of chromatin was preserved when histone H1 was completely cleaved to its trypsin-resistant globular core (see [12]). In other words, the role of histone H1 in maintaining the orienta-

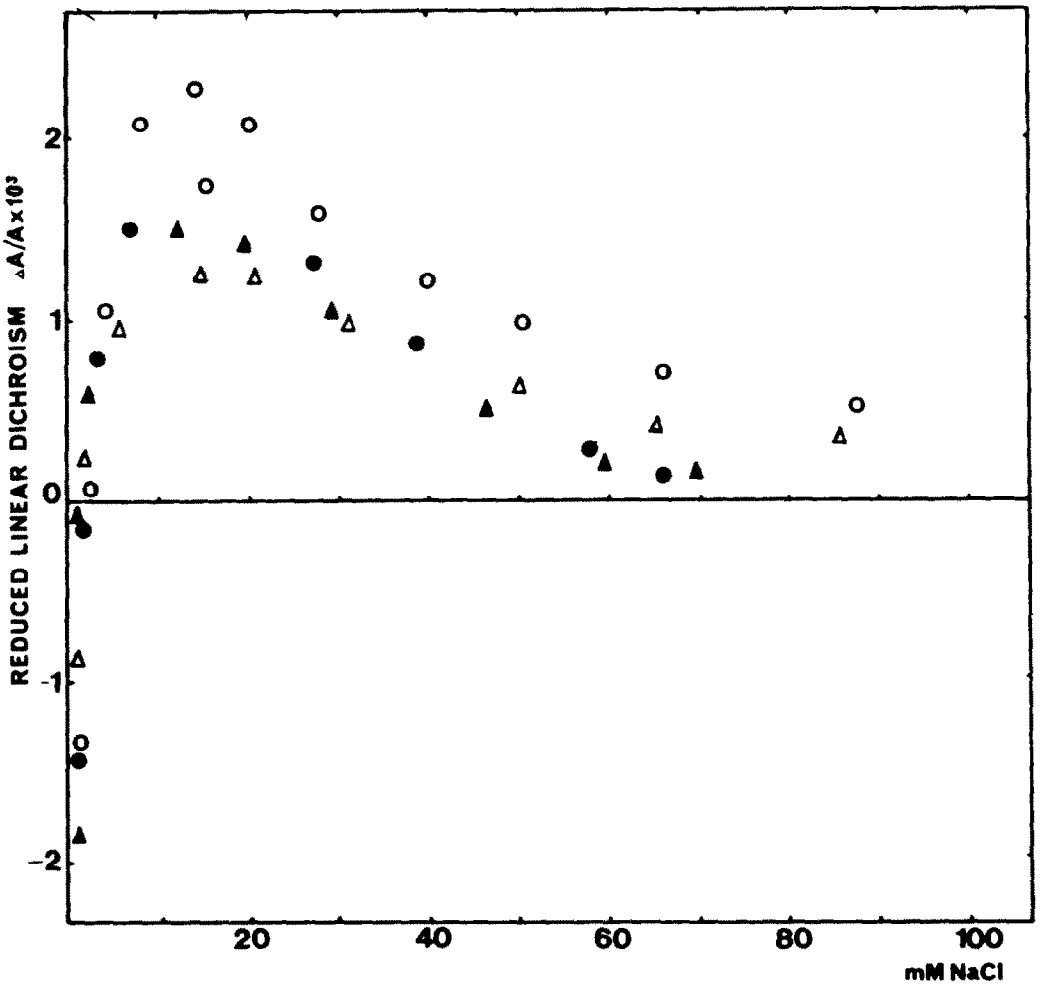

Fig.1. Reduced LD at 258 nm of chromatin isolated from calf thymus (○), chicken erythrocytes (●), sea urchin sperm (△) and Friend erythroleukemia cells (▲).

tion of nucleosomes is accomplished by its globular domain alone.

3. STRUCTURE OF CHROMATIN FIBER: A PROPOSED MODEL

Our attempts to accommodate the flow LD data in a solenoid structure were unsuccessful. The decondensation of the solenoid should be accompanied by a significant reorientation of nucleosomes. The requirement for an invariant orientation of nucleosomal discs upon folding and unfolding of chromatin is satisfied by the 3-dimensional zigzag model presented in fig.2. The repeating unit of this structure is a trinucleosome. The flat faces of nucleosomes are tilted approx. 40° from the fiber axis, which supposed a non-parallel orientation of the adjacent discs along the nucleosomal chain. Twisting and concomitant compression of the zigzag result in a triple helix structure with 0.6–0.7 nucleosomes per nm. Linker DNA is located in the central part of the fiber, exposed to the outside at the grooves between the 3 nucleosomal chains. It is almost straight and, according to Crothers et al. [15], the tilt of the linker from the axis at zero value of the dichroism should be 54.4° instead of 35.6°, a value calculated for the nucleosomal DNA. Our LD studies on chromatin with linkers of different length show that the angle for the tilt of the linker is close to 54°.

The triple helix model easily accommodates some electron microscopic observations and other data obtained by physical methods. The 3-dimensional zigzag correlates well with the zigzag structures seen in micrographs of isolated chromatin [3]. Recently, Subirana et al. [16] reported a 3-dimensional reconstruction of chromatin fiber, calculated from micrographs obtained at different tilt angles. They have found no evidence for regularly repeating interactions among nucleosomes, other than a zigzag pattern.

The studies of oligonucleosomes by electric birefringence [17], light scattering [18] and sedimentation [19] revealed sharp changes in the optical and hydrodynamic properties at the level of the hexanucleosome. It seems, therefore, that the unit of 6 nucleosomes is somehow involved in the formation of chromatin fiber. This was the next point we considered in our model. Although the requirement for an invariant orientation of nucleosomes upon folding-unfolding of chromatin might also be satisfied by a double helix structure, the advantage of the triple helix is that it suggests an explanation of these observations. As seen in fig.3, the smallest number of nucleosomes allow-

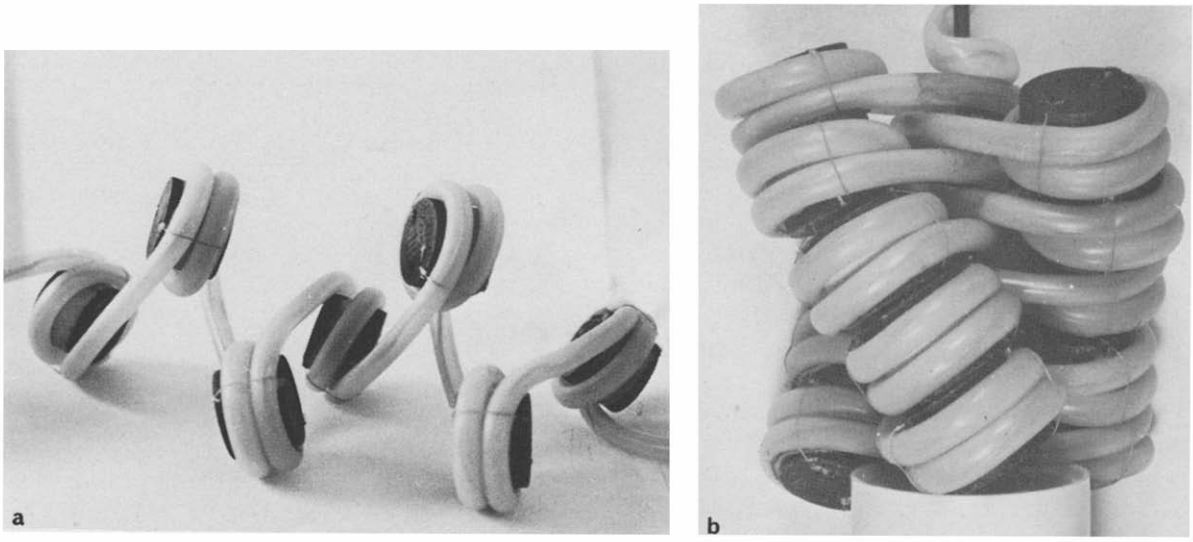

Fig.2. A model of the proposed triple helix structure of chromatin fiber: (a) a 3-dimensional zigzag arrangement of nucleosomes, supposed to take place in low salt, (b) a triple helix structure of chromatin fiber formed upon twisting and concomitant compression of the 3-dimensional zigzag.

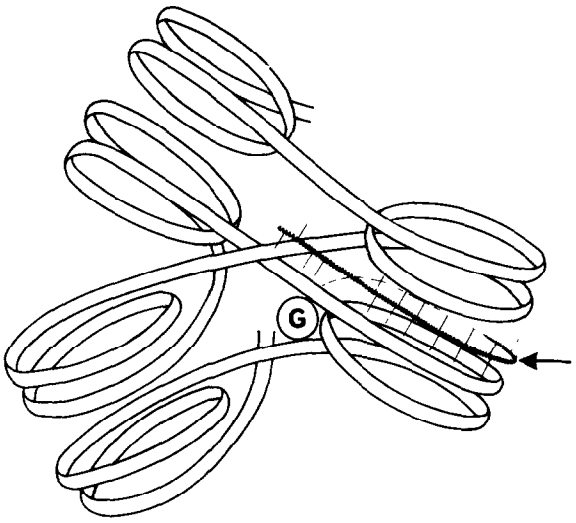

Fig.3. Path of DNA in the 30 nm chromatin fiber according to the triple helix model. The basic unit of 6 nucleosomes is presented together with the locations of the globular part (G) and the C-terminal tail (arrow) of histone H1.

ing all of them to be stacked in pairs is 6. Thus, the hexanucleosome is the smallest unit of the triple helix in which all interactions involved in this structure are presented. It also follows from the model that the diameter of the fiber should be preserved upon folding and unfolding. This feature of the helix received experimental support from recent X-ray scattering studies [20] showing that the diameter of the chromatin fiber in both the folded and unfolded state was 30 nm. Finally, as far as the condensation of chromatin in the triple helix model is accomplished in a manner similar to the folding of an accordion, this process should take place in a very short time. Our data that the condensation of chromatin in 80 mM NaCl, as measured in a stop-flow experiment by following the changes in the intensity of light scattered at 90°, is accomplished in 20 ms (not shown) support such a view.

The role of histone H1 for the integrity of the higher order chromatin structure is well demonstrated, but the location and function of its different domains are not entirely understood. We suppose that the globular part of H1 which is thought to bind at the entry and exit points of DNA in the chromatosome [3,22] serves to fix the mutual orientation of linker DNA. The fixed path of the latter, together with the non-parallel orientation of adjacent nucleosomes result in the formation of a 3-dimensional zigzag with nucleosomal discs tilted from the fiber axis. This possibility is supported by our LD studies on trypsinized chromatin: the role of H1 in maintaining the orientation of nucleosomes is accomplished by its globular domain alone, since the positive dichroism is preserved after cleavage of chromatin with trypsin [14].

The C-terminal region of H1, correctly located in the nucleofilament by the globular part, is the most likely region for the condensation of chromatin [22]. A hypothetical scheme for both the location and function of this domain is presented in fig.3. We suppose that this region in its completely unfolded state is located between the two turns of DNA of N and N+3 nucleosomes, thus decreasing the electrostatic repulsions and favoring stacking. The C-terminal region is highly enriched in lysine, alanine and proline. The ϵ-amino groups of the adjacent lysine residues, being in a *trans* orientation, might work as cross-links between the phosphates of the two stacked nucleosomes thus stabilizing the triple helix. Such a view correlates well with the data of Mirzabekov et al. [23] that histone H1 shows some binding along the whole length of nucleosomal DNA.

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