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Theoretical models of possible compact nucleosome structures

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Abstract

Chromatin structure seems related to the DNA linker length. This paper presents a systematic search of the possible chromatin structure as a function of the linker lengths, starting from three different low-resolution molecular models of the nucleosome. Gay–Berne potential was used to evaluate the relative nucleosome packing energy. Results suggest that linker DNAs, which bridges and orientate nucleosomes, affect both the geometry and the rigidity of the global chromatin structure.

Keywords: Chromatin structure; DNA folding; Low-resolution molecular model; Gay–Berne potential

1. Introduction

Eukaryotic cells contain from 10 to 10^4 millions base pair in a nucleus of a few micrometers in diameter. If all the DNA molecules which constitute a typical eukaryotic genome would be steered, they would span about 3 m in length. An accurate organization of the DNA inside the cell nuclei is therefore necessary.

Packing is due to proteins, which fold DNA, at different levels of organization, into the architecture of chromatin [1]. The first level is well characterized: it is constituted by the nucleosome core particles [2,3], connected together by linker DNAs.

At physiological salt concentration, chromatin is observed in vitro giving a condensed structure called 30-nm fiber, whereas at lower ionic strength, it assumes a more extended conformation.

DNA sequences appear structurally inaccessible and functionally inactive. However chromatin structure should allow the localized unfolding and the subsequent folding of DNA. In particular, it is reasonable to assume that processes, such as replication or transcription, should require a large-scale reorganization of the DNA packing, to allow the binding of the protein factors. However, the knowledge of the internal chromatin structure remains fragmentary.

In the literature, mainly two different models were proposed: the solenoid model [4], in which the linker DNAs are coiled between adjacent nucleosomes, and the zigzag model [5], in which zigzag arrays of nucleosomes form a condensed ribbon generating the 30-nm fiber. In the latter case, linker DNAs are essentially straight.

Zigzag models seem more consistent with the current experimental evidences. Moreover, at low and medium ionic strength, chromatin clearly shows the zigzag arrangement. Finally, DNA linker length exhibits a preferential quantization of its values, preferring integral multiples of the helical repeat [6]. This evidence supports the hypothesis that DNA linkers direct the orientation of consecutive nucleosomes, i.e. if linker length differs of multiples of the helical repeat, consecutive nucleosomes are oriented in the same direction and the corresponding structures are practically equivalent, differing only for the distances between nucleosome cores.

Woodcock and coworkers assumed the nucleosome–linker–nucleosome system as a repetitive unit of the 30-nm fiber and described the observed chromatin structures in terms of the rotation angle between consecutive nucleo-

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somes, a function of the linker length and the linker entry/exit angle (two-angle model). [7–10].

Recently we have found that a three-angle model is a valid proposal for telomeric chromatin, whose structural organization considerably differs from that of the bulk chromatin. To explore a larger conformational space, we considered the dinucleosome as the chromatin repetitive unit, corresponding to introduce two different linker lengths. We found short linkers are compatible with structures resembling those previously proposed for bulk chromatin. In addition, testing different combinations of the DNA linker lengths allowed us to identify a novel compact nucleosomal arrangement [11].

In this paper, we apply our approach also to linker length, which is typical of bulk chromatin, rationalizing the present experimental data and proposing a systematic search for all the possible compact nucleosome structures. In addition, in bulk chromatin, both H1 and histone–tails have been suggested to bridge entering and exiting DNAs together into a stem [9,12–14]. Histone–tails interactions influence DNA distance, contact and parallelism and, consequently, reduce the possible chromatin conformations. Therefore we tentatively considered more realistic models of the entry/exit region.

2. Materials and methods

The initial structures were obtained by repetitions of the fundamental nucleosome–linker 1–nucleosome–linker 2–nucleosome unit. To take into account also the variability of the average helical periodicity, found in natural DNAs, linker length was changed continuously, as proposed in a previous paper [11]. Nucleosomes were generated imposing the correct curvature on 145 bp DNA tracts, in agreement with the crystallographic data [2,3]. Analogously, the nucleosomal DNA periodicity was set equal to 10.2 bp/turn. Interactions between nucleosomes were parameterized using the Gay–Berne potential for oblate ellipsoids [11,15,16]:

$$V(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}}) = 4\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})\left\{\left[\frac{\sigma_0}{r-\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})+\sigma_0}\right]^{12} - \left[\frac{\sigma_0}{r-\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})+\sigma_0}\right]^{6}\right\}$$

where $\hat{\mathbf{u}}_1$ and $\hat{\mathbf{u}}_2$ are unit vectors specifying the axes of the two ellipsoid, $\mathbf{r}$ is the vector distance between their centers, and σ_0=10.3 nm. $\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})$ can be conveniently reduced to $\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2)=\varepsilon_0[1-\chi^2(\hat{\mathbf{u}}_1\cdot\hat{\mathbf{u}}_2)^2]^{-1/2}$, with ε_0=7.57 k_BT [11]. As for $\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})$:

$$\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}}) = \sigma_0\left(1-\frac{\chi}{2}\left\{\frac{(\hat{\mathbf{r}}\hat{\mathbf{u}}_1+\hat{\mathbf{r}}\hat{\mathbf{u}}_2)^2}{1+\chi(\hat{\mathbf{u}}_1\hat{\mathbf{u}}_2)} + \frac{(\hat{\mathbf{r}}\hat{\mathbf{u}}_1-\hat{\mathbf{r}}\hat{\mathbf{u}}_2)^2}{1-\chi(\hat{\mathbf{u}}_1\hat{\mathbf{u}}_2)}\right\}\right)^{-1/2}$$

where the anisotropy parameter is χ=−0.653. Interactions between linker DNAs and between linker DNAs and nucleosomes were considered barely repulsive [11].

3. Results and discussion

In the lack of structural details of the nucleosome entry/exit region, we tentatively considered three different models (Fig. 1). The simplest one (Fig. 1A) resembles the nucleosomal crystallographic structure, where two DNA segments at the termini of the nucleosome are essentially straight [2,3]. Moreover, the entry/exit angle was imposed equal to 40°, a value compatible with those found at physiological ionic strength [9]. In the other models, to mimic the interaction between linker DNAs and H1 globular domain, the nucleosomal DNA curvature was extended to the flanking 10-bp segments at both sides of the nucleosome, increasing the DNA wrapping around the histone core to about 1.9 turns. Afterwards, we imposed that DNA linkers wind around each other up to half a turn [13] over a distance of 20 bp. This value was chosen as an approximate average between the stem length found in mononucleosomes (about 32 bp) [13] and in minicircles (about 10 bp) [12,14]. Finally, DNA linkers form an angle of 40°, laying in planes parallel (Fig. 1B) or perpendicular (Fig. 1C) to the nucleosomal average plane.

Fig. 2 shows the diagrams of the chromatin packing energy as a function of the two linker lengths, varying within a range of 11 bp around the chicken erythrocyte nucleosomal length (~66 bp). Most of the map points corresponds to not favored conformations; however, very narrow minima are present. Contrary to the case with short

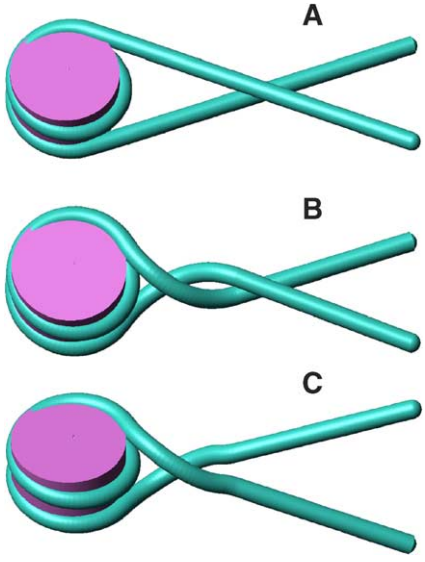

Fig. 1. Representation of the geometrical structure of the nucleosome models. A: Without stem. B: With the stem and the linker DNAs parallel to the nucleosomal average plane. C: With the stem and the linker DNAs laying on planes perpendicular to the nucleosomal average plane.

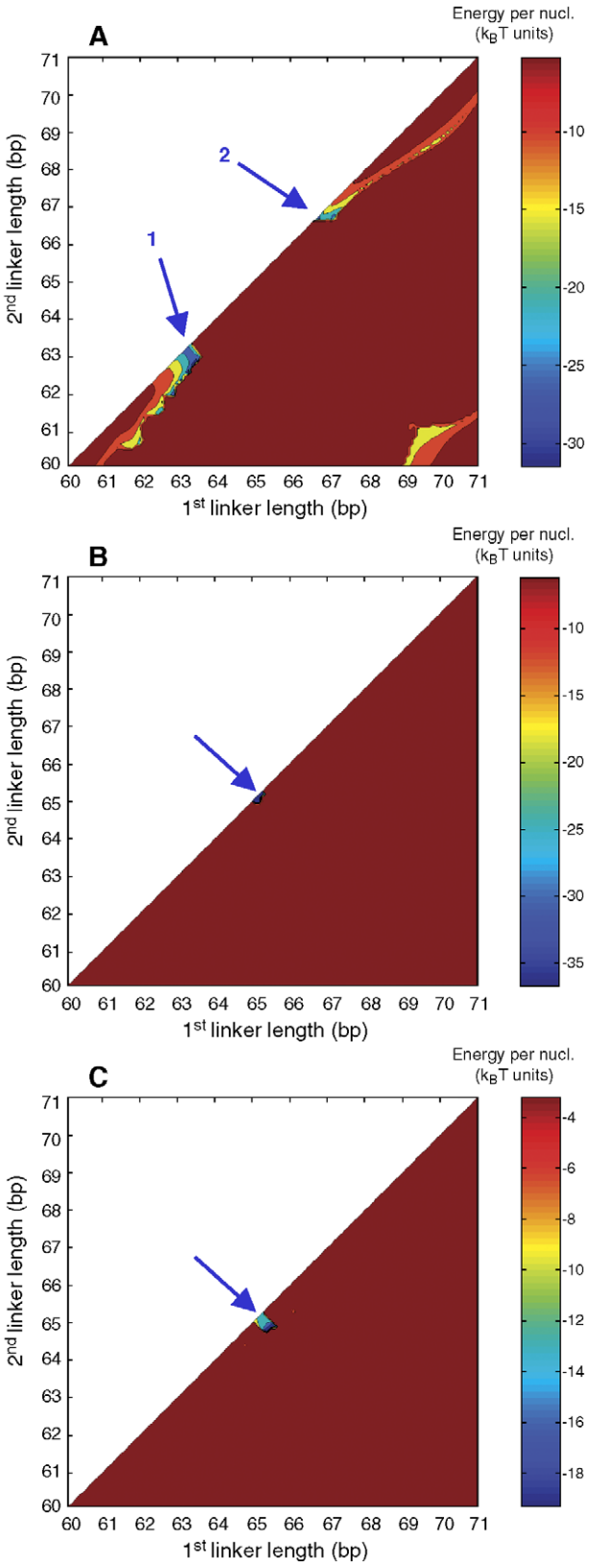

Fig. 2. Diagram of the chromatin packing energy per nucleosome as a function of the two DNA linker lengths. Positive energy values have been cut off as they correspond to not favored conformations. Arrows indicate the positions of the energy minima.

linker lengths [11], all favored conformations are really close to the diagonal.

In the case of model A, two minima were found. Unexpectedly, the most stable (-31.4 k_BT/nucleosome) corresponds to the structure with the lower nucleosomal linear density (Fig. 3A'). The second minimum, having an energy of -22.6 k_BT/nucleosome, is more compact and resembles the chromatin model proposed by Woodcock and coworkers [5,7,9] (Fig. 3A").

In the case of models B and C, favored chromatin conformations are strongly reduced to just one minimum (with energy equal to -36.7 and -19.3 k_BT/nucleosome, respectively), with DNA linker lengths of about 65 bp (Fig. 2B and C). However, the global shape of the structure strongly depends on the stem model. In particular, the structure corresponding to model B (Fig. 3B) resembles the structure in Fig. 3A", even if it is more compact. On the contrary, the structure corresponding to model C (Fig. 3C) does not appear to have a good nucleosome packing.

However, in the framework of model approximations, it should be noted that energy stabilization is highly cooperative in all the possible structures (model B giving the best result). Therefore, it is possible that some twisting deformations occur to linker DNAs during fiber formation, which could be compensated by the favorable nucleosome

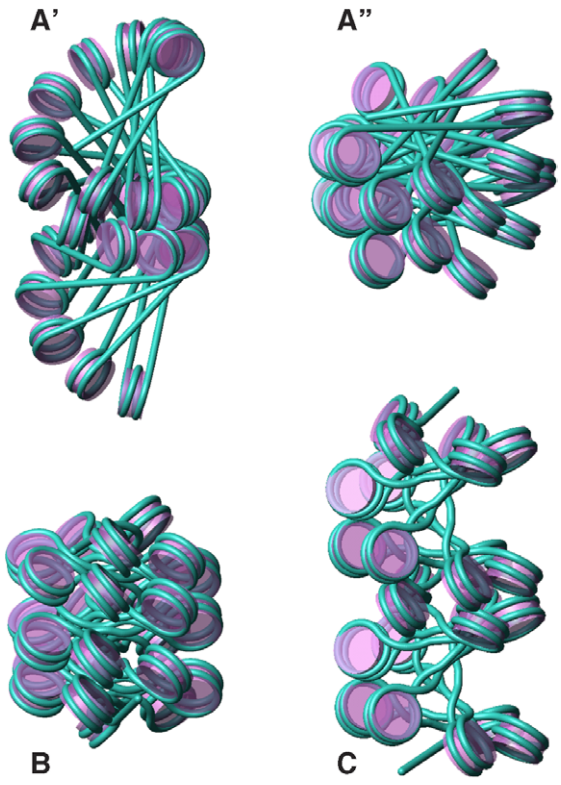

Fig. 3. Visualization of the molecules with the lowest energy, corresponding to the energy minima in the maps in Fig. 2. A': Structure corresponding to model A with l_1=63.2 bp, l_2=62.5 bp. A": Structure corresponding to model A with l_1=l_2=66.6 bp. B: Structure corresponding to model B with l_1=65.0 bp, l_2=64.9 bp. C: Structure corresponding to model C with l_1=65.4 bp, l_2=64.8 bp.

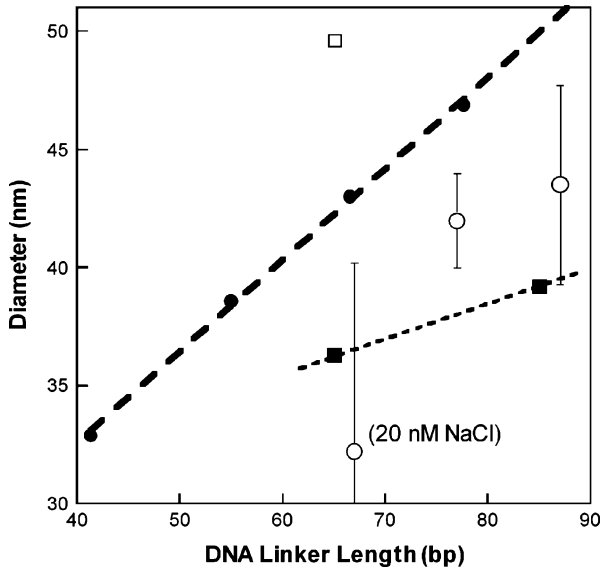

Fig. 4. Calculated fiber diameter as a function of the linker length for the three models in Fig. 1: ●, model A (2nd minimum); ■, model B; □, model C. Connecting lines are guides for the eye and have no physical meaning. Open circles represent experimental data from cryoelectron microscopy [17]. Experimental diameter reported for isolated chicken erythrocyte chromatin fibers at 20 nM NaCl showed a wide range of values, probably depending on the high sensitivity of the sample to the ionic composition of the medium [17].

interaction energy. This eventually corresponds to having additional possible linker length combinations.

Finally, Fig. 4 reports the value of the fiber diameter as a function of the linker length for the three models we considered. The calculated values were compared with experimental data from cryoelectron microscopy. Indeed, the presence of chromatin fibers in frozen hydrated nuclei made it possible to obtain measurements of fiber diameter that were not compromised by the effects of fixation, dehydration, or embedding [17].

In principle, if linker length differs by multiples of the helical repeat, the corresponding fiber structures are practically equivalent. However the requisites for stem formation prevent from having too short linkers in the case of models B and C. In addition, model C clearly produces structures with too large fiber diameter with respect to the experimental data and was not further considered. On the contrary, model B seems the most appropriate to describe chromatin structure.

4. Conclusions

Three- or two-angle models have the great advantage to describe chromatin geometry in terms of a few parameters that characterize the single nucleosomal structure. The chromatin fiber is obtained as an ordered repetition of conformational equivalent units, In addition, specific sequence-dependent effects could be treated as deviations from the basic model.

However, the presence of both H1 and the histone tails affects the geometry of the entry/exit DNAs and, consequently, DNA folding into the 30-nm fiber.

Bypassing the lack of structural details, we have built three different nucleosome models to test how much they could affect the global structure and stability of the 30-nm fiber. Our results show that DNA folding, like that in Figs. 3B and C, seems the most appropriate to produce compact structures, independently of the stem presence. Much more relevant is the orientation of the linker DNAs, as demonstrated by the comparison between Figs. 3C and D. On the contrary, the stem presents strongly narrow energy minima and seems to be essential for the mechanical rigidity of the fiber. However, it is clear that for a more straightforward comprehension of the DNA folding in chromatin, a deeper knowledge of the geometry of the stem region can not be eluded.

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