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Adaptive elastic properties of chromatin fiber

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Abstract

Chromatin is a complex of DNA and specific proteins forming an intermediary level of organization of eukaryotic genomes, between double-stranded DNA and chromosome. Within a generic modeling of chromatin assembly, we investigate the interplay between the mechanical properties of the chromatin fiber and its biological functions. A quantitative step is to relate the mechanics at the DNA level and the mechanics described at the chromatin fiber level. It allows to calculate the complete set of chromatin elastic constants (twist and bend persistence lengths, stretch modulus and twist–stretch coupling constant), in terms of DNA elastic properties and geometric features of the fiber. These elastic constants are strongly sensitive to the local architecture of the fiber and we argue that this tunable elasticity might be a key feature in chromatin functions, for instance in the initiation and regulation of transcription. Moreover, this analysis provides a framework to interpret micromanipulation studies of chromatin fiber and suggests further experiments involving intercalators to scan the tunable elasticity of the fiber. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chromatin is a protein–DNA complex forming the basic material of chromosomes of all eukaryotic organisms and strikingly conserved during evolution [1]. It provides an intermediary level of organization between the underlying DNA double-helix and the whole cell machinery. Chromatin achieves essentially two fundamental functions: it compacts efficiently DNA into the cell nucleus, and it compacts it operationally in order to allow targeted gene expression. We propose a mechanical approach of these

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two questions. It suggests that the chromatin structure might be selected according to its elastic properties, which might be exploited during transcription by the various enzymes at work during this process, for instance to locally condense (gene silencing) or decondense (gene activation) the fiber.

2. Chromatin assembly

We introduce a basic geometric modeling of chromatin assembly, which allows to incorporate acknowledged data on DNA structure, its bend and twist persistence lengths, and data on the nucleosome structure into an analytically tractable framework. Chromatin is composed of a double-stranded DNA molecule wrapped from place to place around protein cores (histone octamers). Focusing on the native case where these cores are fixed on the DNA, we model separately the nucleosomes, i.e., the DNA-wrapped histone cores, and the linkers, i.e., the naked DNA segments connecting the nucleosomes.

Since we are looking for generic properties of the chromatin fiber, originating from its assembly, sequence effects are ignored. We suppose that all the linkers have the same number n of base pairs; it corresponds to currently observed phased nucleosomes [2]. A posteriori, we may argue that self-organization of the fiber architecture leads to such a regular positioning of the nucleosomes in order to get proper structural and elastic properties. In fact, we need this hypothesis to be satisfied only locally, over a few linkers. Linker DNA is described as a non-extensible semi-flexible polymer within the continuous worm-like-chain model supplemented with twist elastic degree of freedom [3]; it thus involves two persistence lengths, respectively, of bend ($A = 53$ nm) and twist ($C = 75$ nm), the given values corresponding to 10 mM NaCl [4]. Linkers are straight in absence of applied constraints.

Nucleosome structure is now well-known thanks to high-resolution crystallography data [5]. The net effect of a nucleosome on the DNA trail can be described as a rigid kink connecting two successive linkers. The slope of the DNA path on a nucleosome being fixed (angle 4.5°), a single angle Φ between entering and outgoing linkers thoroughly prescribes the geometry. This picture allows an effective modeling of various physico-chemical effects, for instance electrostatic repulsion between linkers and its variable screening when the ionic strength is increased. It accounts also for histone H1 binding in the neighborhood of linker entry/exit points as well as histone tails influence and its variation upon posttranslational covalent modifications of these tails [6–9]. We here ignore interactions between nucleosomes, which is valid as soon as the internucleosomal distances are larger than the interaction range; in any cases, the model describes the geometric contribution to chromatin elastic behavior originating from the DNA elastic properties.

Our model, quite similar to the so-called two-angle model of Woodcock et al. [10], amounts to build the chromatin fiber nucleosome after nucleosome. The rotational positioning of a nucleosome with respect to the previous one is entirely prescribed by the twist angle of the DNA linker connecting them. In the relaxed state, it is equivalently prescribed by the linker length n (in bp) since the twist angle is then

precisely equal to $\tau = 2\pi n/10.6$ (recall that there is 10.6 bp per DNA turn). Each step of the assembly is described analytically, but we implemented this model within a Maple program in order to handle an arbitrary number of nucleosomes and to perform a quantitative analysis of the chromatin fiber geometric properties.

3. Structural properties

The implementation of the above model straightforwardly gives all the geometric properties of the chromatin fiber. Homogeneity of the local architecture (uniform values for n and Φ) enforces a helical symmetric structure for the fiber; we shall speak of “chromatin superhelix” (SH), and denote \vec{A} its axis. A complete study of the symmetry properties and quantities relevant to the mechanics of the fiber has been presented in a previous paper [11]. We here only underline the high structural sensitivity of the fiber, evidenced in Fig. 1: for various values of n and Φ , we obtain the whole variety of structures suggested from experimental studies [1,12–14]. These qualitative results support our minimal two-parameter modeling, since it is sufficient to recover the actual diversity of chromatin structures. A noticeable point, evidenced in Fig. 2, is the fact that the diameter remains about 30 nm whatever the structure; the so-called 30 nm-fiber observed experimentally might thus correspond to far different structures. Excluded volume has to be taken into account to discard unrealistic structures; we determined a sufficient condition to avoid steric hindrance, which is shown in Fig. 2.

One of the conclusions of our structural study is the fact that the connection between the microscopic parameters and the SH geometric characteristics is too complex and multivariate to get small-scale informations from structural observations of the fiber; we now turn to the analysis of its mechanical properties.

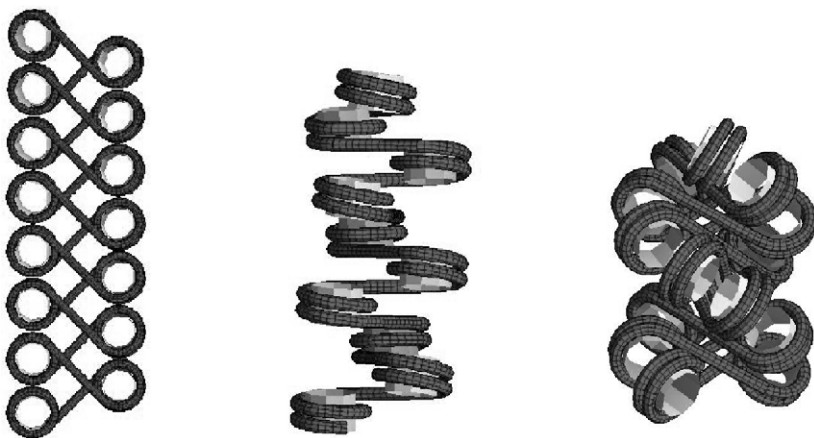


Fig. 1. Varying the linker length n and the entry/exit angle Φ recovers all the structural diversity observed experimentally, for instance ribbon ($\Phi = 90^\circ$, $n = 58$ bp), columnar structure ($\Phi = 50^\circ$, $n = 42$ bp) or double-helical structure ($\Phi = 60^\circ$, $n = 56$ bp).

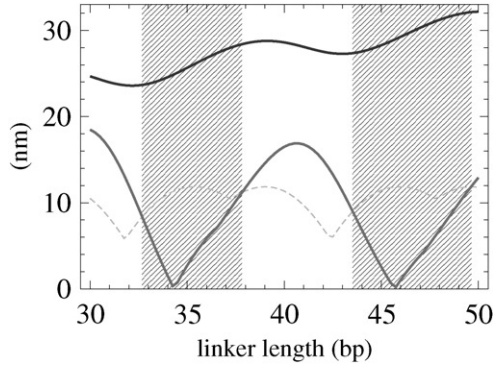


Fig. 2. Diameter of the chromatin fiber (upper curve) and its pitch \mathcal{P} (lower curve). The thin line gives a lower bound \mathcal{P}_c for the pitch above which no steric hindrance occurs within the fiber, in contrast to the dashed regions where $\mathcal{P} < \mathcal{P}_c$.

4. Elastic properties

In order to obtain the leading order of the elastic constants of the fiber, we merely consider a straight fiber with cylindrical symmetry. Describing the elastic properties of the chromatin fiber is thus nothing but a problem of spring mechanics [15]. Nevertheless, the architecture of this “spring” is much more complex than a simple helical coiling and we expect that the detailed structural features of the chromatin assembly strongly influence the behavior at the fiber scale. Our modeling allows us to compute analytically the elastic constants describing the linear response of the fiber when a force \vec{F} and a torque \vec{M} are applied at its ends, hence to investigate quantitatively how the elastic response of the fiber varies with its relaxed structure. Note that this structure is itself controlled by the “microscopic” structural parameters n and Φ .

Our computation lays on the identification of the elastic energy of the fiber when a force \vec{F} and a torque \vec{M} are applied at its ends and the sum of the elastic energies of its linkers (bend and twist energies) in such a constrained configuration. This identity follows from the absence of internucleosomal interactions and to our focus on the linear response regime (no modification of the fiber architecture at this order); it amounts to restrict to the elastic contribution originating from linker elasticity within the relaxed chromatin structure. The first step is then to relate the “macroscopic” stresses (\vec{F}, \vec{M}) to the distribution of stresses along linker DNA. We denote $\vec{f}_j(s)$ the force and $\vec{m}_j(s)$ the torque exerted at the point $P_j(s)$ of linker j (with arclength s) by the upstream part of the fiber. We focus on the universal, rotationally symmetric behavior of the fiber. Writing standard equilibrium equations of spring mechanics, and using the linear response hypothesis to simply sum up the effects of the different stresses applied to the fiber (stretching force, bending torque and torsional torque), we obtain:

$$\begin{aligned} \vec{f}_j(s) &= \vec{F}, \\ \vec{m}_j(s) &= \vec{M} - [\vec{O}_j(s)P_j(s)] \wedge \vec{F}, \end{aligned} \tag{1}$$

where $O_j(s)$ denotes the orthogonal projection of $P_j(s)$ onto the SH axis \vec{A} . It gives the relation between the global stresses \vec{F} and \vec{M} exerted at the fiber ends and the local stresses $\vec{f}_j(s)$ and $\vec{m}_j(s)$ experienced at the linker level, at each point of the DNA path. The term $[\vec{O}_j(s)P_j(s)] \wedge \vec{F}$ reflects the involvement of the fiber architecture in the expression of the local torque $\vec{m}_j(s)$. This set of equations is the core of our analysis since it allows to perform explicitly the change of description level, namely passing from the DNA level to the fiber level and reciprocally.

As we restrict to the description of harmonic elasticity, the coefficients of \vec{F} and \vec{M} in the elastic energies of the linker will be computed within the relaxed SH. We underline that we do not need to compute the constrained shape of the linkers to describe the linear response of the fiber to applied force and torque. The cylindrical symmetry of the fiber ensures that \vec{F} is directed along the SH axis \vec{A} at equilibrium. \vec{M} decomposes into a bending torque \vec{M}_b and a torsional torque M_t according to $\vec{M} = \vec{M}_b + M_t \vec{A}$. The well-established elastic energy of a linker [3,4] can then be expressed as a function of F , M_t and M_b thanks to (1); it yields a quadratic expression

$$\mathcal{E}_{linker} = a_s F^2 + a_t M_t^2 + 2a_{ts} M_t F + a_b M_b^2 \quad (2)$$

which is to be identified with the elastic energy \mathcal{E}_{fiber} of a length D of chromatin fiber, where D is the distance between two successive nucleosomes measured along the SH axis (projected distance). Plugging in the quadratic expression $\mathcal{E}_{linker}(F, M_t, M_b)$ the linear response ansatz relating the stresses F , M_t and M_b experienced by the fiber and its strains u (relative extension), Ω (twist rate) and ρ (local curvature):

$$\begin{pmatrix} F \\ M_t \\ M_b \end{pmatrix} = \begin{pmatrix} \gamma & k_B T g & 0 \\ k_B T g & k_B T \mathcal{C} & 0 \\ 0 & 0 & k_B T \mathcal{A} \end{pmatrix} \begin{pmatrix} u \\ \Omega \\ \rho \end{pmatrix} \quad (3)$$

naturally embeds the fiber and its elastic behaviour within a current continuous model known as the “extensible worm-like rope” [16], in which the density of elastic energy writes, as a function of the canonical variables u , Ω and ρ :

$$\varepsilon_{fiber} = \frac{k_B T \mathcal{A} \rho^2}{2} + \frac{k_B T \mathcal{C} \Omega^2}{2} + \frac{\gamma u^2}{2} + k_B T g \Omega u. \quad (4)$$

\mathcal{A} is the bend persistence length of the fiber, \mathcal{C} its twist persistence length, γ its stretch modulus (dimension of a force) and g the twist–stretch coupling constant (no dimension). Note the expected vanishing of the twist–bend and stretch–bend coupling (for symmetry reasons [16]). We may now express the elastic constants of the fiber in terms of the coefficients a_b , a_t , a_{ts} and a_s , obtained as functions of the DNA elastic constants and fiber geometry, by a term-wise identification of the coefficients of u^2 , Ω^2 , $u\Omega$ and ρ^2 in the following identity:

$$\begin{aligned} \mathcal{E}_{fiber} &\equiv D\varepsilon_{fiber} = D \left(\frac{k_B T \mathcal{A} \rho^2}{2} + \frac{k_B T \mathcal{C} \Omega^2}{2} + \frac{\gamma u^2}{2} + k_B T g \Omega u \right) \\ &= \mathcal{E}_{linker}[F(u, \Omega), M_t(u, \Omega), M_b(\rho)] \end{aligned} \quad (5)$$

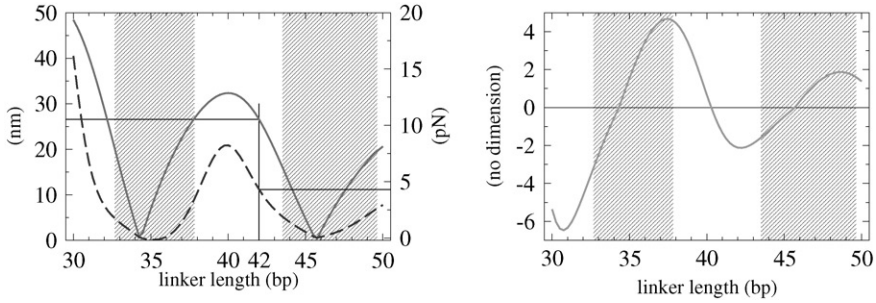


Fig. 3. Elastic constants of the chromatin fiber versus linker length n (in bp) for $\Phi = 50^\circ$. A similar sensitivity when n varies is observed for other values of Φ , or when varying Φ at fixed n . (a) Bend persistence length \mathcal{A} (solid line) in nm and stretch modulus in pN (dashed line); here is shown the effective modulus $\gamma_{\text{eff}} = \gamma - k_B T g^2 / \mathcal{C}$ which is involved in force-extension curves [16,11] hence directly measurable. Our computations predict that \mathcal{A} and the twist persistence length \mathcal{C} are almost equal, both close to the pitch \mathcal{P} of the SH (Fig. 2). Experimental measurements of Cui and Bustamante [17] yield a value about 30 nm for \mathcal{A} and 5 pN for γ_{eff} (horizontal lines). (b) Twist–stretch coupling constant g (no dimension). Note that the vanishing of g corresponds to a change of chirality for the fiber.

from which follows analytical expression [11] of the elastic constants \mathcal{A} , \mathcal{C} , g and γ . We present in Fig. 3 the results for $\Phi = 50^\circ$ (high salt concentration, with histone H1, i.e., physiological conditions) when the linker length is varied. The curves evidence a strong sensitivity of elastic coefficients with respect to the fiber structure, here controlled by n . This sensitivity originates in the sensitivity of the SH pitch: accumulation of turns in a spring leads to a highly flexible accordion-like behavior. It is to note that the persistence length \mathcal{A} and \mathcal{C} are almost identical, both close to the SH pitch and smaller than the corresponding persistence lengths A and C of DNA. More strikingly, the stretch modulus of the chromatin fiber, around 5 pN, is to be compared to that of DNA ($\gamma_{\text{DNA}} \approx 1100$ pN): whereas DNA can be seen as non-extensible, chromatin is highly extensible; in particular, entropic and enthalpic elastic regimes (well-separated in the case of DNA) will overlap. We finally underline a key feature: the twist–stretch coupling constant, reflecting the chirality of the chromatin fiber, exhibits steep variations and sign changes when the linker length is varied. The same twisting of the fiber will either condense or decondense it according to the relaxed structure.

5. Experimental implications

A first issue involving fiber elasticity is to describe the linear elastic response of the fiber to global stresses, i.e., a force and a torque applied at its ends. This issue refers to micromanipulations in which a single chromatin fiber is pulled [17] (and possibly will be twisted). Describing the fiber within the extensible worm-like rope model, Eq. (4), yet thoroughly investigated in the context of DNA by Marko [16], allows an interpretation of the force-extension curves in terms of geometric and mechanical parameters of the fiber. Experimental results of Cui and Bustamante [17] are in good agreement with our predictions (note that their measure rests on relaxation curves, for

which there is no interaction between nucleosomes, consistently with our modeling). Indeed, they measured a bend persistence length $\mathcal{A} \approx 30$ nm and a stretch modulus $\gamma \approx 5$ pN, which are obtained simultaneously in our modeling for $n \approx 42$, or any integer multiple of DNA pitch; we there recover the nucleosome phasing observed in biological experiments [2].

Our study should now be supplemented by investigating the effect of a dispersion in the values of n and Φ . It is likely that the dispersion will induce a kind of averaging. Continuity arguments lead us to expect the sensitivity of the structural and elastic properties to remain—although possibly smoothed out—together with its possible relevance in biological regulation. Work is in progress on this question.

To circumvent this limitation, we suggest to take advantage of the interplay between linker intercalation and the chromatin fiber mechanics. The twist change $\Delta\tau$ achieved in the linker by intercalator molecules (for instance with ethidium bromide in vitro) amounts to shift the linker length by $\Delta n = 10.6 \times \Delta\tau/2\pi$. Plotting the various elastic constants as a function of Δn should provide similar curves as those shown in Fig. 3, however, smoothed out. We therefore suggest that it should be possible to scan experimentally these curves by varying continuously the concentration of intercalators added in the buffer when performing pulling fiber experiments.

6. Biological relevance

Another issue, directly relevant to the in vivo functioning of chromatin, is to investigate both the effect of DNA–protein binding on the fiber structure, and the effect of fiber stresses on the linker DNA–protein binding. Our theoretical approach gives a framework to such studies by relating the structure and mechanics at the DNA scale and those at fiber scale.

On one hand, our approach allows to describe the response of the fiber to local, internal stresses as those created by intercalators, groove-binding proteins, or any induced change in the fiber assembly at the linker scale. Mechanical sensitivity is likely to provide efficient switches for processes involving a conformational transition of the fiber, for instance the decondensation required prior transcription or, at the opposite, fiber compaction currently associated with gene silencing [18]. A possible tuning mechanism lays on the variation of Φ , controlled in particular by the presence of linker histone, salt concentration and histone tails binding affinities.

On the other hand, protein–DNA interactions involved in gene-regulated subcellular events take place within chromatin fiber. In consequence, they are likely to be influenced, if not directly regulated, by chromatin structure and by the stresses generated in the linkers when the chromatin fiber is somehow constrained. In particular, we expect such an involvement of chromatin structure and multilevel mechanical properties to be at work in epigenetics and gene imprinting [19].

7. Conclusion

The chromatin scale is precisely the scale of nanomechanics: at this scale, we expect a strong and direct interplay between the biological functioning, monitored by various

enzymes, and the mechanical properties of the chromatin fiber. Our complete and quantitative analysis gives clues to discuss how the elastic properties of the chromatin fiber might at the same time favor DNA compaction into the chromosomes and allow local and controlled decondensation of chromatin involved in gene expression.

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