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Mechanically stretching single chromatin fibers

Research Article

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Summary

We have used the recently developed MAC Mode Atomic Force Microscope (AFM) that operates in aqueous solution to mechanically stretch single chicken erythrocyte chromatin fibers. The fibers contained the full complement of histones, or, alternatively, were depleted of linker histones. The AFM was used to produce the so-called force curves, by monitoring the cantilever deflection (proportional to force) as the distance between the AFM tip and the sample was experimentally manipulated. To that end, the AFM tip was pushed into the chromatin sample and then withdrawn, to mechanically stretch the fiber that was physically adsorbed to the tip. Pulling of the chromatin fiber produced complex sawtooth-like patterns of peaks that were characterized by unexpectedly large forces, in the range of several hundred picoNewtons. The distribution of forces in the linker histone-containing and linker histone-depleted fibers was remarkably different, possibly indicating that linker histone binding significantly changes the mechanical properties of the chromatin fiber.

I. Introduction and theoretical background

In the eukaryotic nucleus, processes such as transcription, replication, and repair that use DNA as a template take place in the context of chromatin. The machineries performing these reactions have to gain access to the DNA by initially disrupting the higher-order structure of the chromatin fiber (van Holde and Zlatanova, 1996), and by further moving the nucleosomes out of the way of the enzymes. The mechanisms involved in rendering the DNA accessible are not well understood. The notion that the histone octamers that form the protein core of the nucleosomal particles are removed from the DNA by an applied tension-based mechanism has attracted considerable attention, especially after the discovery that movement of the polymerase along the DNA during transcription by itself can create the necessary tension. It has been suggested, and later proven experimentally, that transcription is accompanied by the formation of relatively high levels of positive supercoiling in front, and negative supercoiling in the wake of the passing polymerase (Liu and Wang, 1987). In such a scenario, the polymerase itself may be considered as a molecular motor creating forces to help evict the histone octamer from the DNA.

In order to gain insight into the forces that maintain the integrity of chromatin structure, we made an attempt at directly measuring these forces with the help of the Atomic Force Microscope (AFM). Chromatin fibers were physically adsorbed onto glass coverslips, the AFM tip was pushed into the chromatin sample, and then pulled away. The deflection of the cantilever was then monitored as a function of its distance from the surface, producing the so-called force-extension curves. The cantilever deflection can be used to directly determine forces by multiplying its magnitude by the spring constant of the cantilever (Hooke's law). Such curves have been widely used in recent years to study intermolecular interaction forces between pairs of interacting molecular partners (Moy et al, 1994; Florin et al, 1994; Lee et al, 1994a, b; Dammer et al, 1996; Allen et al, 1997). In such curves, the interaction between the molecular partners (e.g. antigen-antibody) is revealed as hysteresis between the approach and retraction curve (see **Figure 1**, for a schematic of such curves). The magnitude of tip deflection in the so-called adhesion peak

is taken as an indication of the magnitude of interaction rupture forces. More recently, force curves have been used to determine the forces maintaining the native conformation of single-chain biological polymers, such as multi-domain proteins (Rief et al, 1997a; Oberhauser et al, 1998) or polysaccharides (Rief et al, 1997b; Marszalek et al, 1998). Stretching of multi-domain proteins produces curves with multiple, sawtooth-like discontinuities thought to reflect unfolding of individual folded domains. Such discontinuities occur at points during stretching at which the cantilever restoring force F exceeds the molecular interaction forces that are responsible for the integrity of the domain structure. Thus, the magnitude of the vertical jumps in the force curves can be used to estimate interaction forces.

II. Imaging and stretching chromatin fibers

A. Native or linker histone-depleted chicken erythrocyte chromatin fibers

In our analysis of chromatin, we first imaged the fibers and then monitored the force-extension curves, in low ionic strength buffers that favor the extended fiber conformation (Zlatanova et al, 1998). Imaging was important in two aspects: first, we could be sure, on the basis of the density of the material on the surface, that in each individual pulling event we were stretching single fibers; second, the morphology observed and the quantitative measurements done on imaged fibers helped us to interpret the results. Chicken erythrocyte chromatin fibers containing all histones were imaged both in air (**Figure 2a**) and in buffer (**Figure 2b**). In both cases, the fibers had an irregular three-dimensional appearance, similar to the previously published tapping mode images (Leuba et al, 1994). Typically, the force curves taken on such material were rather complex, exhibiting multiple snap-off peaks separated from each other at certain intervals (**Figure 3**).

Similar experiments were also performed on linker histone-depleted fibers. Such fibers are known to lose their three-dimensional organization when imaged in the AFM (Leuba et al, 1994; Yang et al, 1994), and resemble artificial chromatin constructs containing only a piece of DNA to which core histones were added (Allen et al, 1993). The linker histone-depleted fibers also produced multi-peak curves, superficially similar to those obtained on 'native' fibers.

Figure 4 presents the distribution of forces measured from large sets of force curves. As can be seen, the forces in the linker histone-depleted fibers are rather broadly distributed, with a mean ~ 400 pN. In contrast, the native fibers had a narrower distribution, centered around 100 pN.

In order to analyze the complex force curves obtained on chromatin fibers, we switched to artificial chromatin constructs that are of a known length and contain a known number of nucleosomes (on average), more or less regularly spaced along the DNA length. Such artificial constructs were obtained by in vitro reconstitution of tandemly repeated cloned fragments of the 5S rRNA gene (twelve repeats of a 208 bp sequence) from the sea urchin *Lytechinus variegatus* with purified core histones.

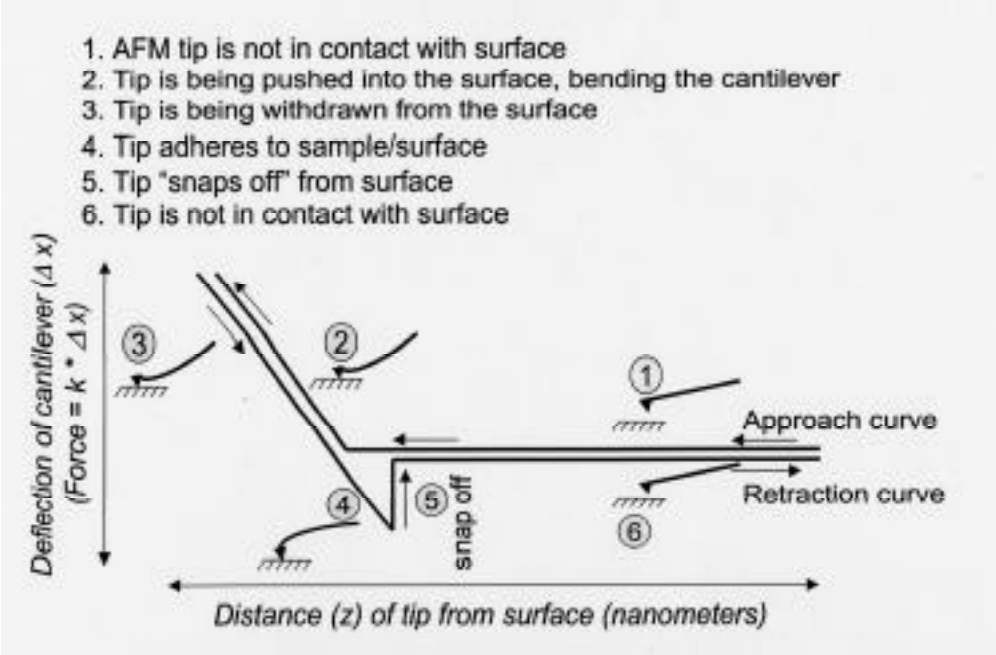

Figure 1. Schematic of a typical contact mode force curve.

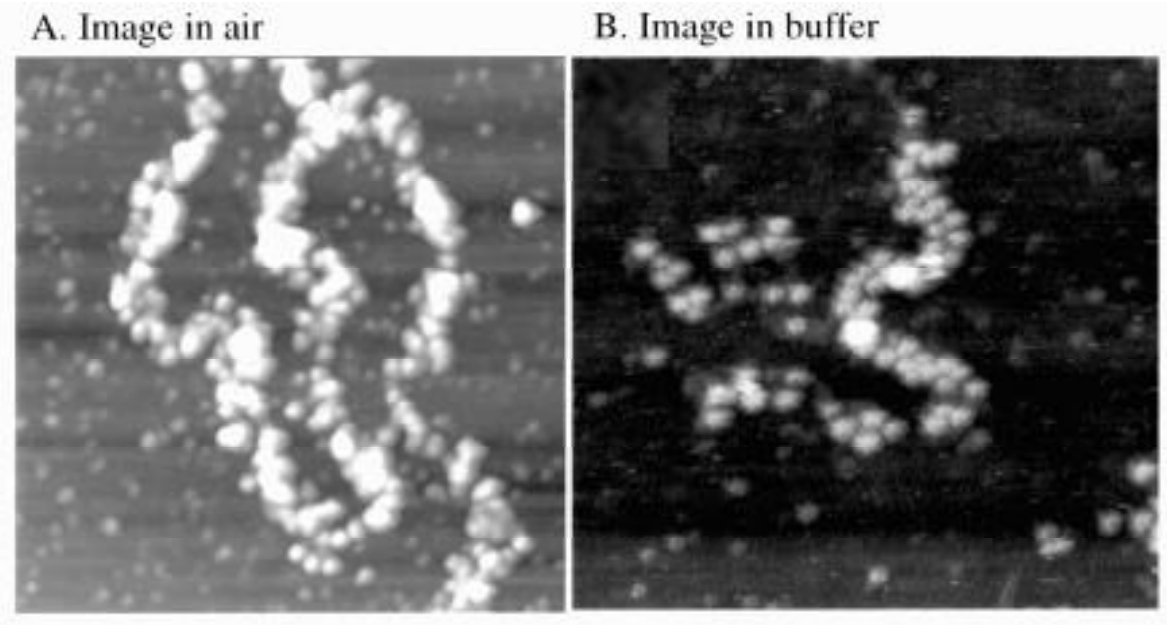

Figure 2. Chicken erythrocyte chromatin fibers imaged in air (A) and low ionic strength buffer (5 mM triethanolamine, pH 7.0, 0.1 mM EDTA) (B). (B) is adapted from Leuba and Bustamante, 1999. The height of features above the surface is denoted in different shades of gray, in a range from 0 to 15 nm, with the higher features being lighter. Scan size 500 nm x 500 nm. Chicken erythrocyte chromatin fibers were obtained as described in Leuba et al. (1994).

This nucleosomal array, first introduced in the laboratory of R. Simpson (Simpson et al, 1985), is widely used in the chromatin structure field in view of the regularity of its structure. For these constructs, the force distribution was similar to that of the linker histone-depleted chromatin fiber (Leuba, Zlatanova, Karymov, Liu, R. Bash, D. Lohr, R. Harrington, Lindsay, in preparation). The relative simplicity of this system, well characterized in biochemical terms, will hopefully help us interpret the chromatin data presented here.

III. Analysis of possible structural transitions in chromatin fibers during mechanical stretching

How do we interpret these results? What are the events occurring during stretching of the chromatin fibers that give rise to the sawtooth pattern of peaks? While a detailed interpretation is not possible at this point, the pulling curves clearly reflect nucleosomal-related events.

Such an assertion is based on two main observations: (i) When naked DNA was deposited and pulled under the same experimental conditions, no peaks were observed (including the first 'adhesion' peak), indicating that DNA does not stick to the surface under these conditions. (ii) There was a good correlation between the number of (presumably connected) nucleosomes in the images taken under liquid of linker histone-lacking fibers and the number of peaks observed in the retraction curves (Leuba et al, in preparation). (The number of nucleosomes in images taken in air were close to the numbers expected from the known loading of the fibers with nucleosomes; in contrast, the number of nucleosomes in buffer images was always much less than the known number on the fibers, indicating that the attachment of the fiber to the surface in liquid did not involve the entire length of the fiber).

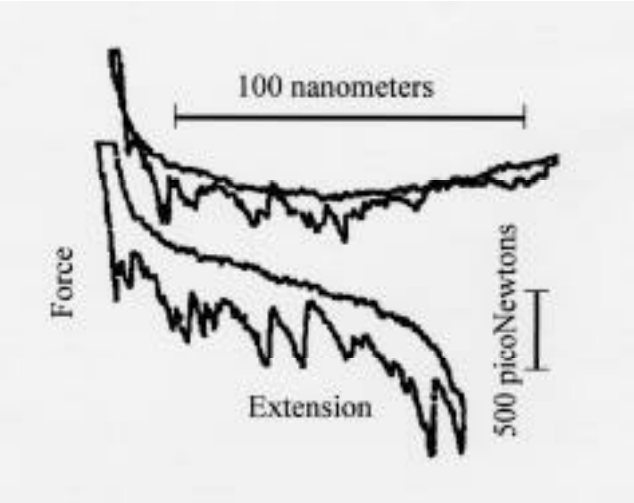

Figure 3. Typical force curves obtained on pulling chicken erythrocyte chromatin fibers. In each set of curves, the upper curve is obtained upon moving the AFM tip downward, the lower upon withdrawing the tip.

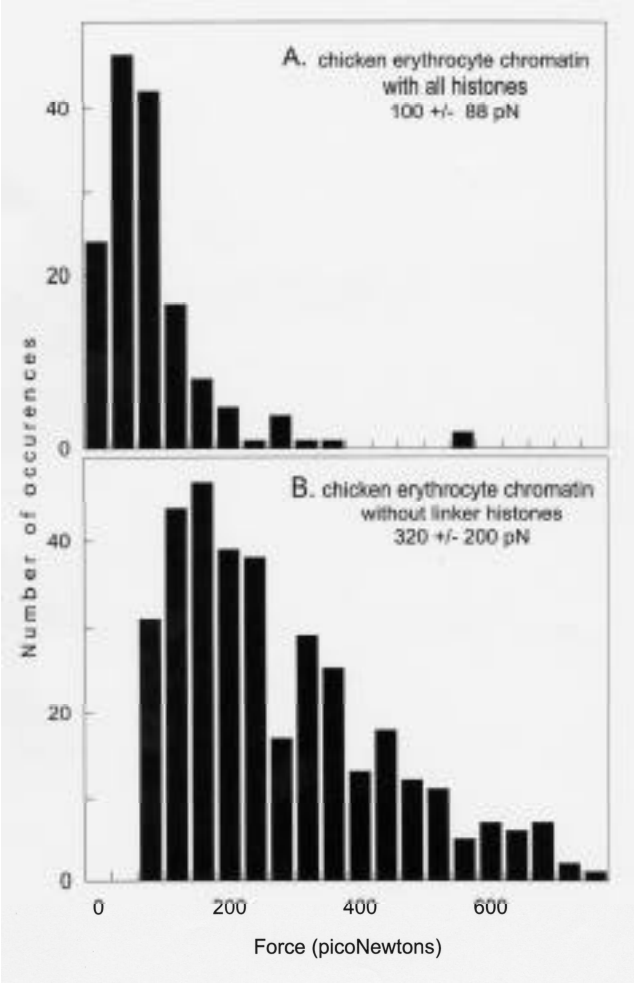

Figure 4. Frequency distribution of forces measured from chromatin force curves: (A) native fibers, (B) linker histone-depleted fibers. Chicken erythrocyte chromatin fibers were depleted of linker histones as described in Leuba et al. (1994).

In principle, the peak events may reflect either (or both) of two things: detachment of individual nucleosomes from the surface during the pulling-off, or unraveling (unwrapping) of the DNA from around the histone octamer. It is not possible to distinguish between these two processes on the basis of the available chicken chromatin fiber data.

Further analysis will obviously be needed to interpret these complex chromatin fiber curves. Even in the absence of a strict interpretation, our data show remarkably large forces upon mechanically stretching chromatin fibers. Moreover, the unexpectedly large changes in force upon removal of linker histone may reflect significant differences in the mechanical properties of linker histone-containing and -depleted chromatin fibers.

Our findings are rather surprising in view of the large forces observed upon application of tension to the chromatin fiber. These forces are very close to the forces actually measured to break DNA (Bensimon et al, 1995; Noy et al, 1997; Rief et al, 1999). It must be noted, however, that in our pulling experiments we are far from thermodynamic equilibrium conditions since we are applying the pulling force at rather large loading rates (typically between $4x10^4$ and $4x10^6$ pN/second). It has been recently demonstrated that the forces monitored in pulling experiments are strongly dependent on the pulling rate (Evans and Ritchie, 1997; Rief et al, 1997a; Merkel et al, 1999). We believe that further improvements in instrumentation will allow us to apply much lower loading rates, thus approaching thermodynamic equilibrium conditions.

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Jordanka Zlatanova (left) with Paola Caiafa
(At the 1999 Conference in Crete)