# Distinct levels in the nanoscale organization of DNA-histone complex revealed by its mechanical unfolding

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Mechanical unfolding of nanoscale DNA-histone complex, using an atomic force microscope, shows a stepwise disassembly of histones from the nucleosome. A quantitative analysis of the rupture jump statistics and the length released per jump reveals insights into the possible histone contacts within the octamer complex. The measured ruptures correlate with the breakage of multiple contacts that stabilize the histone octamer. These results provide a mechanistic basis by which stepwise disassembly of histone proteins may result from an external force exerted by the adenosinetriphosphate (ATP) dependent chromatin remodeling machines to access regulatory sites on DNA. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2728031]

DNA in eukaryotic cells is packaged to varying degrees within the nucleus by histone and other nuclear proteins into higher order chromatin structure.[1] Individual nucleosomes are composed of histone octamers (two H2A-H2B dimers and one $(H3\text{-}H4)_2$ tetramer) held together by protein-protein interactions and further wrapped around by about 146 base pairs of DNA by electrostatic interactions.[2] These histone-histone interactions are mediated through noncovalent interactions and are tuned by the physicochemical modifications of the unstructured tail residues of histones.[3] Force-dependent disintegration of chromatin structure is thought to be the prime mechanism, by which mechanoenzymes bring about conformational changes of nucleosome assembly to access regulatory sites on DNA.[4]

Recent progress in understanding the physics of DNA-histone and histone-histone interactions elucidates their importance in the mechanical stability of genome organization and function.[5,6] Histone octamers can be broken into its constituents, thus perhaps altering the energy barriers for the translocation of adenosinetriphosphate (ATP) dependent molecular motors on DNA.[7] In addition, dynamic equilibrium between histone octamers and its constituent monomers has been reported in fluorescence resonance energy transfer and fluorescence recovery after photobleaching studies, suggesting the role of conformational fluctuations in displacing nucleosomes and in accessing regulatory sites.[8–10] By pulling the two ends of the DNA in a nucleosome array, the forces required to dislodge histone octamers from DNA loops were measured.[11–15] Further, unfolding of large scale chromatin organization has yielded the overall elastic properties of chromosomes.[16] However, in the above studies, the forces that stabilize the histone-histone contacts at the level of mononucleosomes have not been addressed.

In this work we present dynamic force spectroscopy based measurements that capture histone interactions in mononucleosomes. The nucleosomes were purified from HeLa cells for the atomic force microscopy (AFM) experiments, as described in the supplementary material. The freshly prepared mononucleosome samples were incubated on aminopropyltriethoxysilane (APTES)-glutaraldehyde (GD) coated mica for 15 min so that the –NH2 groups of the proteins are bound covalently to the GD on the mica surface. Samples were then settled onto the mica for imaging. The samples were washed thoroughly to remove any free-floating proteins and imaged in phosphate-buffered saline (PBS) buffer using commercial AFM setup PICOSPM-II from Molecular Imaging Inc. AFM images of mononucleosome samples were taken in tapping mode [Fig. 1(a)]. The average height of these nucleosome core particles was found to be 8 nm [Fig. 1(b)]. Using these images of mononucleosomes we position our cantilever on an individual histone octamer, identified by its height, and the resultant rupture force profile of octamer dissociation was recorded. For strong adhesion with the surface, both the glass/mica substrate and the AFM cantilevers (Molecular Imaging) were APTES coated by vapor deposition method.[17] The sample substrate and AFM cantilevers were then incubated in 1 mM glutaraldehyde for 15 min and then washed with MilliQ water followed by PBS. For control experiments, the mononucleosomes were fixed in solution using 2% paraformaldehyde for 30 min. The paraformaldehyde fixes the histone proteins to each other.

Figure 2 shows a typical force-extension curve of a single histone octamer. Cantilever deflection was measured

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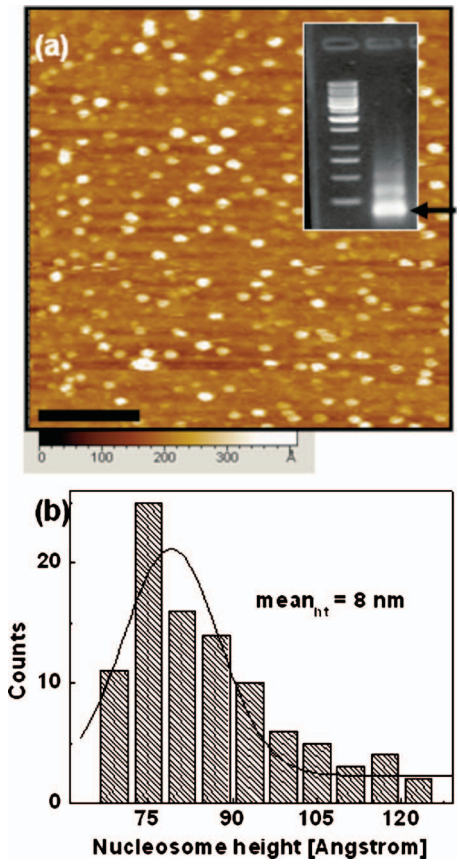


FIG. 1. (Color) (a) AFM image of the mononucleosomes. Inset: Agarose gel showing the ladder pattern of DNA after digestion of chromatin with micrococcal nuclease. The arrow shows the mononucleosome band at ~150 bp. (b) Height distribution of mononucleosomes obtained from the AFM images.

as a function of distance of tip from the sample surface. We see the histone octamer dissociated in a stepwise fashion, typical of protein unfolding experiments.[18] At the end of the stretch cycle, the cantilever returns to its free position. Since the histone octamer is covalently bound between the mica and AFM tip, the observed sawtooth pattern, see inset (i), Fig. 2, indicates sequential disruption of strong histone-histone interactions in individual histone octamers. As a control, force extension on paraformaldehyde-fixed mononucleosomes abolished multiple jumps almost completely, see inset (ii), Fig. 2. Rupture jumps are defined by a shift in deflection value of more than $3\sigma$ (standard deviation) within four bins (<20 ms for the slowest rate). The slow releases, which happen over many bins, are not considered jumps. All data analysis was done by custom written software in LABVIEW (National Instruments).

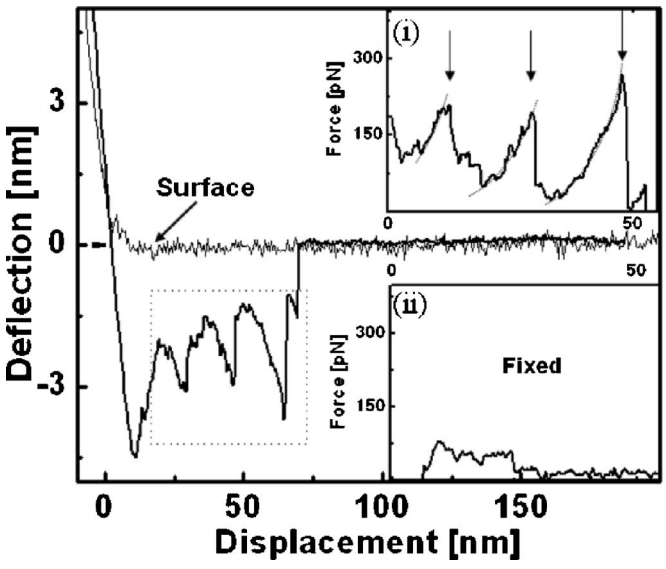


FIG. 2. Typical force-extension curve of mononucleosomes and from the background surface as a control. [Inset (i)] High resolution data from the dotted rectangle depicting the stepwise disassembly. The dotted lines are guides for the eye. [Inset (ii)] Rupture curves from fixed mononucleosomes.

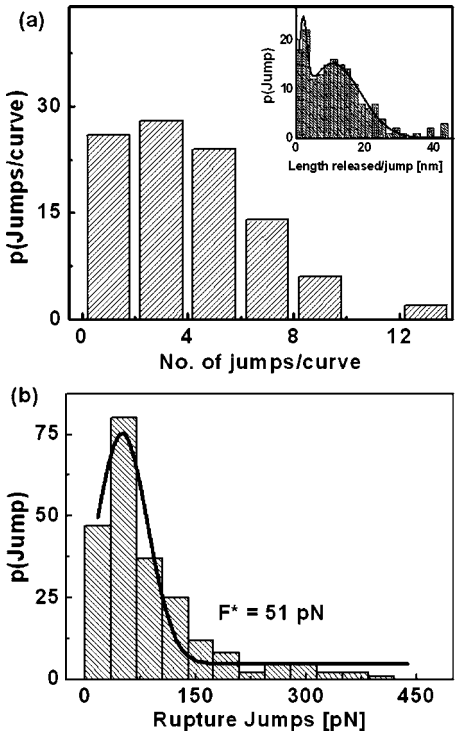


FIG. 3. (a) Number of ruptures per force-extension curve on mononucleosomes. (Inset) Polymer length released per rupture jump extracted from distance between adjacent jumps. (b) Rupture jump statistics of mononucleosomes at loading rate of $10^{-7}$ N/s.

Several rupture patterns were analyzed and the number of rupture events encountered in a given force curve per nucleosome were plotted as a function of their frequency of occurrence [Fig. 3(a)]. Figure 3(a) shows that the average number of jumps for histone octamers ranges from 1 to 7. In inset to Fig. 3(a), we plot the length released between two jumps in any given force-extension curve. Apart from the initial smaller peak, which we assign to probable nonspecific adhesion, the most probable length released during the force-extension cycles of mononucleosomes is ~10 nm. These experiments show a good correlation with the crystal structure data in terms of the number of histone-histone contact regions reflected in the number of jumps observed. Different motifs of histone proteins H2A, H2B, H3, and H4 contact each other at various locations to form the octamer. These histone-histone contact regions (at least ten points of contact[4]) as seen from the crystal structure data need to be broken in order to disrupt the octamer, and this is possibly reflected in the number of rupture events observed in each force-extension curve. The length released in each jump is ~10–15 nm, which is much shorter than the total contour length of a histone protein (~130 amino acids corresponding to about 40 nm). It is likely that the histone proteins do not

unfold completely, but rather partially open up to a length corresponding to ~10 nm. The abolishment of these jumps in paraformaldehyde-fixed mononucleosomes supports this hypothesis. Statistics of the rupture forces are plotted in Fig. 3(b) and the most probable rupture force (F*) is found to be ~51 pN for a loading rate of $10^{-7}$ N/s. The loading rate dependence of the rupture force is shown in the supplementary material. This force is relatively higher than the force required to release the nucleosome core particle from the DNA,[10–14] indicating that protein-protein interactions stabilizing the histone octamer are stronger than DNA-protein interactions.

The histone proteins H2A, H2B, H3, and H4 interact with each other based on the helix-loop-helix interactions. The assembly of a stable nucleosome core depends on the initial $(H3\text{-}H4)_2$ tetramer formation which can form a stable complex with ~120 bp DNA. Dimers of (H2A-H2B) bind to both sides of the tetramer and extend the wrapping of DNA within the nucleosome to >160 bp. Our observations suggest a model where histone proteins are linked to each other in a nucleosome core particle. When anchored from one end of a surface, a force on the opposite end of the octamer unravels it into its individual component histones. The different histone octamer components are bound strongly to each other via multiple interaction sites, and as the cantilever is retracted, these interacting regions give way resulting in a sawtoothed pattern of the rupture profile. This suggests a means by which mechanoenzymes may sequentially displace histone proteins from the core particle, thus subtly changing the local energy barriers required to access naked DNA. Indeed, emerging biochemical evidence suggests that the histones, which form the octamer core of the nucleosomes, are released in a stepwise fashion by the chromatin remodeling enzymes.[19] On applying an external force of about ~20–50 pN chromatin remodeling enzymes can break open the nucleosome structure, thus making it easy for the regulatory machinery to access naked DNA. The discrete unfolding steps observed in our experiments may have implications in understanding the electrostatics of DNA-histone assembly,[2] their positioning,[20] and its disassembly by external forcing.[21] In addition, the mechanisms that underlie histone-DNA assembly are beginning to provide clues to self-assemble DNA polymer based three-dimensional nanoscale structures.[22]

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