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Dynamics of Nucleosomes Assessed with Time-Lapse High-Speed Atomic Force Microscopy

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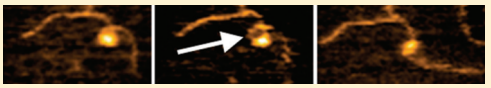
Supporting Information

ABSTRACT: A fundamental challenge of gene regulation is the accessibility of DNA within nucleosomes. Recent studies performed by various techniques, including single-molecule approaches, led to the realization that nucleosomes are quite dynamic rather than static systems, as they were once considered. Direct data are needed to characterize the dynamics of nucleosomes. Specifically, if nucleosomes are dynamic, the following questions need to be answered. What is the range of nucleosome dynamics? Is a non-ATP-dependent unwrapping of nucleosomes possible? What are the factors facilitating the large-scale opening and unwrapping of nucleosomes? In previous studies using time-lapse atomic force microscopy (AFM) imaging, we were able, for the first time, to observe spontaneous, ATP-independent unwrapping of nucleosomes. However, low temporal resolution did not allow visualization of various pathways of nucleosome dynamics. In the studies described here, we applied high-speed time-lapse AFM (HS-AFM) capable of visualizing molecular dynamics on the millisecond time scale to study the nucleosome dynamics. The mononucleosomes were assembled on a 353 bp DNA substrate containing nucleosome-specific 601 sequence. With HS-AFM, we were able to observe the dynamics of nucleosome on a subsecond time scale and visualize various pathways of nucleosome dynamics, such as sliding and unwrapping to various extents, including complete dissociation. These studies highlight an important role of electrostatic interactions in chromatin dynamics. Overall, our findings shed new light on nucleosome dynamics and provide a novel hypothesis for the mechanisms controlling the spontaneous dynamics of chromatin.



The dynamics of the nucleosome core particle (NCP), a fundamental unit of chromatin, is a key property of chromatin, allowing proteins involved in chromatin transcription or replication accessibility to DNA wrapped around the histone core.[1] Remodeling systems provide the accessibility of DNA within nucleosomes,[2] but this process can be facilitated by the inherent dynamics of nucleosomes.[3] Single-molecule approaches of various types were instrumental in characterization of nucleosome dynamics. These methods include fluorescence resonance energy transfer,[4−10] single-molecule probing,[11,12] and atomic force microscopy (AFM) imaging.[13−16] Single-molecule fluorescence and time-resolved techniques revealed that nucleosomes undergo local dissociation of DNA in the absence of remodeling proteins,[8,17−20] and this process occurs on the subsecond time scale.[19] Earlier, AFM imaging was used to characterize chromatin structure at the nanoscale level.[21−23] The AFM sample preparation is so gentle that AFM allows the study of chromatin structure omitting the rather traditional glutaraldehyde fixation procedure of the sample.[24] Note in this regard, recent studies using time-lapse observations allowed direct observation of the dynamics and unwrapping of nucleosomes.[25,26] The unwrapping of nucleosomes proceeds from the ends of the particle, resulting in unwrapping of DNA regions as large as dozens of base pairs. This process may lead to a complete unfolding of nucleosomes and dissociation of the histone core from the complex. Nucleosome dissociation occurs in the absence of ATP-dependent protein systems involved in chromatin remodeling, suggesting that the inherent dynamics of nucleosomes can contribute to the chromatin unwrapping process. However, because of the slow data acquisition rate of time-lapse AFM techniques, many dynamic events are missing. The advent of high-speed AFM (HS-AFM)[27] and recent advances in this technique[28] made it possible to directly visualize dynamic processes, on the millisecond time scale, of site-specific DNA−protein complexes[29,30] and nucleosomes,[31] allowing the identification of novel mechanisms of protein−DNA dynamics.

Here we applied HS-AFM to image nucleosome dynamics. The data demonstrate that nucleosomes undergo spontaneous unwrapping, occurring on the subsecond time scale. Although site exposure is the predominant mode of nucleosome dynamics and unwrapping, transient sliding of the nucleosome also takes place. The sliding process is facilitated in the presence of the zwitterion detergent, CHAPS, allowing large-range nucleosome translocation and unwrapping. The unwrapping dynamics depends on the DNA sequence facilitating the sliding pathway for sequences with low specificity for binding histone cores. The role of intermolecular electrostatic interactions in chromatin dynamics, dissociation, and unwrapping and the interaction with remodeling systems are discussed.

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MATERIALS AND METHODS

Preparation of Nucleosomal DNA. DNA used for the nucleosome assembly is similar to that used in previous studies.[26] DNA substrate was generated by polymerase chain reaction using plasmid pGEM3Z-601 as a template, which encodes a high-affinity nucleosome positioning sequence 601.[32] The 601 sequence (147 bp) was located inside the 353 bp DNA fragment with 127 and 79 bp arm DNA, so that the mononucleosome reconstituted with the DNA substrate has two DNA arms that differ in length by 1.6-fold. A schematic for the DNA substrate is shown in Figure S1 of the Supporting Information.

Histone Octamer Assembly and Purification. Histone octamers were assembled as described in refs 26 and 33. Octamers were separated from tetramer and dimer fractions via size-exclusion chromatography (SEC) with Superdex 200 PC 3.2/30 columns (GE Healthcare) at 4 °C. SEC fractions were analyzed for purity and histone stoichiometry using sodium dodecyl sulfate−polyacrylamide gel electrophoresis. Fractions containing histones H2A, H2B, H3, and H4 in approximately equal ratios were pooled and concentrated by centrifugation at 10000*g*.

Nucleosome Refolding. Nucleosomes were prepared as described previously.[26,34] Briefly, histone octamers and DNA containing the nucleosome positioning sequence were mixed in equimolar concentrations in 2 M NaCl and kept for 30 min at room temperature. A series of dilutions was prepared by using 10 mM Tris-HCl to produce final NaCl concentrations of 1, 0.67, and 0.5 M. Diluted samples were kept at 4 °C for 1 h before being dialyzed against one change of volume of 0.2 M NaCl overnight. Nucleosomes were concentrated using a Microcon centrifugal filter device (molecular weight cutoff of 10000) at 7000*g* for 10 min at 4 °C and dialyzed against one change of 200 mL of buffer containing 10 mM HEPES-NaCl (pH 7.5) and 1 mM EDTA for 3 h at 4 °C.

Atomic Force Microscopy. The sample preparation procedure for high-speed AFM was modified to accommodate the instrument design requirements. Freshly cleaved mica was modified with a 167 μM solution of 1-(3-aminopropyl)silatrane (APS) to make APS-mica.[26,35,36] APS-mica is a positively charged surface that binds DNA primarily electrostatically allowing AFM observation of segmental mobility of DNA in the free state and in protein−DNA complexes;[26,37,38] 1.5 μL of an APS solution (167 μM) was placed on the freshly cleaved mica (a disk ~1.5 mm in diameter glued to the sample stage) for 30 min. The surface was rinsed with 3 μL of milli-Q water 10 times and with 3 μL of "imaging" buffer containing 10 mM HEPES (pH 7.5) and 4 mM $MgCl_2$ five times. A droplet of 1.5 μL of solution containing the nucleosome sample was applied to the APS-mica surface for 5 min and then rinsed with the imaging buffer. The nucleosome sample was prepared from the stock solution by dilution into 10 mM Tris-HCl (pH 7.5) and 4 mM $MgCl_2$ buffer.

The high-speed AFM instrument used in this work was developed by the Ando group[27,28] and manufactured by RIBM (Tsukuba, Japan). The nominal spring constant of the cantilevers was 0.1−0.2 N/m, and the resonance frequency in water was between 400 and 1000 kHz. The scanning rate in various experiments varied between 1 and 5.5 frames per second.

We used BL-AC10DS-A2 cantilevers (Olympus) modified by the electron beam deposition method and sharpened by using a plasma etcher.[27,28,39]

The processing of individual frames of HS-AFM files and conventional AFM images was performed using Femtoscan Online (Advanced Technologies Center, Moscow, Russia).

RESULTS

Nucleosome Unwrapping Imaged with HS-AFM. Previously, with the use of standard time-lapse AFM, we were able to observe spontaneous unwrapping of nucleosomes.[26,36] Here we applied the ability of HS-AFM to image dynamics on the millisecond time scale to assess nucleosome dynamics with substantially higher temporal resolution. The nucleosome samples were deposited on an APS-mica surface without a drying step (see Materials and Methods). A few frames taken with a scanning rate of 1 frame per second are shown in Figure 1. We observed that initially, highly wrapped

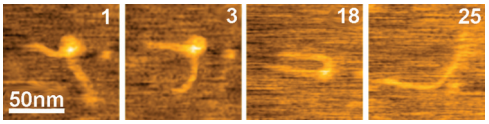

Figure 1. Selected AFM images illustrating nucleosome unwrapping, obtained at the capture rate of 1 frame per second.

nucleosome particles (frame 1) start unwrapping shortly (frame 3), leading to a complete unwrapping of DNA, with a histone core remaining bound internally after 18 s (frame 18), followed by the dissociation of the histone core (frame 25). This process is similar to our previous observations with the use of time-lapse AFM with the slow data acquisition capability;[26,36,40] however, the current data reveal that the dynamics takes place on the subsecond time scale and the entire unwrapping of the nucleosome occurs in <1 min.

Transient states of the unwrapping process were detected at the elevated scanning speed of HS-AFM. Figure 2 shows the data obtained with a scanning rate of ~5.5 frames per second. A full set of the data, assembled as a movie file (Movie 1), is available in the Supporting Information. Images shown in set A of Figure 2 reveal that initially a tightly overwound nucleosome (frame i) is loosening (frame ii), followed by asymmetric unwrapping in which only one arm (the bottom one) increases in length, whereas the size of the top arm does not change visually (frames iii and iv). This conclusion is supported by the length measurements shown in Figure 2B; the vertical dashed lines indicate times corresponding to images in each frame in Figure 2A. The length of the long upper arm (black squares) remains constant, whereas the bottom short arm increases in length, with the process occurring in a two-step fashion (indicated with arrows). The first step appears at ~3.5 s during which ~19 bp are added to the arm. At 8.5 s, the second step is observed, with the unwrapping elongating the left arm by ~40 bp. Note that both transitions take place on a time scale of <1 s.

The plot in Figure 2C shows the data obtained for a similar asymmetric process for another nucleosome, in which the long arm becomes longer and the short arm remains essentially unchanged. See Movie 2 in the Supporting Information for the images. The length measurements for the short arm reveal complex nucleosome dynamics, in which the short arm undergoes shortening and elongation processes at 7.5 and 9.5 s,

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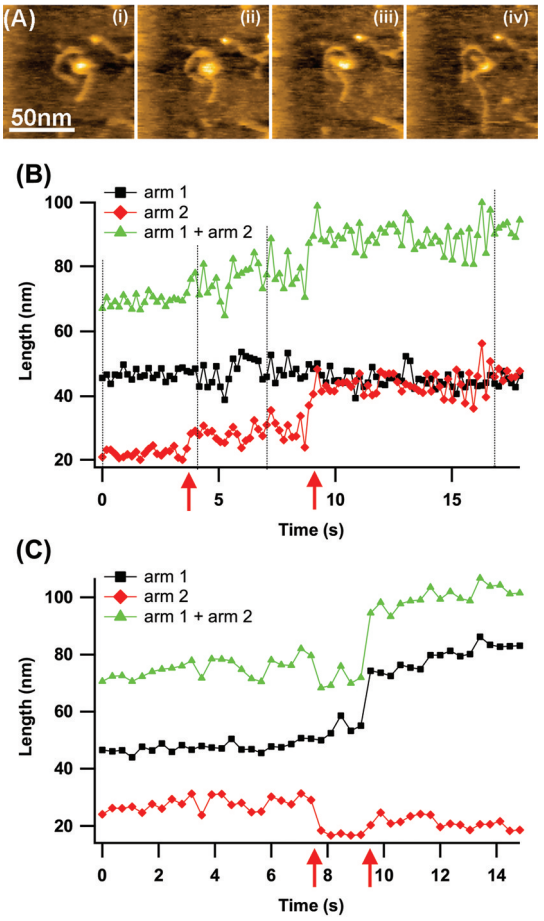

Figure 2. Time-lapse HS-AFM visualization of nucleosome unwrapping in which only one end of the DNA unwraps. (A) Set of AFM images for the nucleosome in which the bottom arm increases in size; the initial image (i) and three frames with the same nucleosome captured after 4.2 (ii), 7.1 (iii), and 17 s (iv) are shown. (B) Time dependence of the lengths of the upper arm (arm 1, black squares) and bottom arm (arm 2, red diamonds) along with the overall DNA length (green triangles). The dashed lines correspond to the times the images shown in A were acquired, and the red arrows correspond to the stepwise unwrapping process. (C) Time dependence for another nucleosome in which only the length of arm 1 increases. See panel B for the code. The data were obtained with a scan rate of 1 frame per 353 ms.

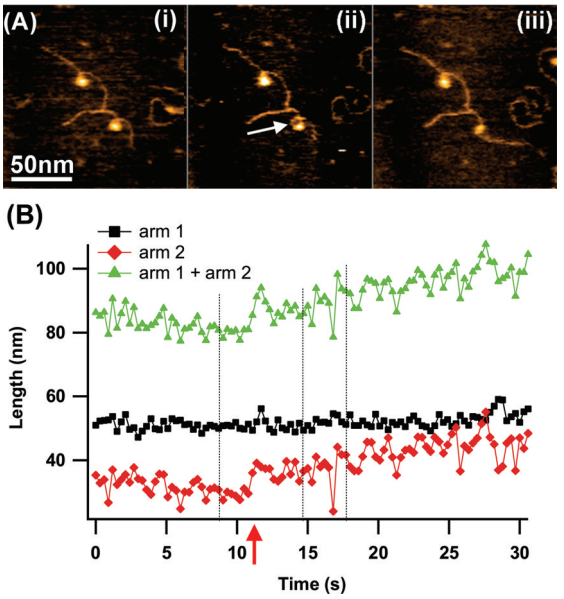

Figure 3. Loop formation and unfolding. (A) Set of images corresponding to 8.7 (i), 14.7 (ii), and 17.1 s (iii). In panel ii, the positions of the DNA dissociation and unlooping events are indicated with a white arrow. (B) Length measurements for the looping and loop unfolding process. The lengths of the left (arm 1) and right (arm 2) arms and the total DNA length are shown with black squares, red diamonds, and green triangles, respectively. In panel B, the dashed lines correspond to the image acquisition times shown in panel A. The scan rate is 1 frame per 301 ms.

respectively. As in the previous graph, the unwrapping process involving dozens of DNA base pairs occurs fast, on the subsecond time scale.

Images in Figure 3A show details for the unwrapping process (imaging rate of 3.3 frames per second). Initially, the wrapped nucleosome (frame i) unfolds generating a loop (frame ii), followed by the loop unfolding (frame iii). The quantitative analysis of these data is shown in Figure 3B. The vertical dashed lines in the figure indicate the times of the images shown in Figure 3A. The long arm (squares) remains unchanged, whereas the short arm increases in length sharply at ~12 s, followed by a gradual increase, reaching a length close to that of the left arm. The large set of images assembled as a movie file (Movie 3) is provided in the Supporting Information.

Reversible Octamer Sliding in Specific Nucleosomes. Although unwrapping is the primary process by which nucleosomes can spontaneously dissociate, HS-AFM allowed us to observe sliding. Three successive frames of a nucleosome undergoing sliding are shown in Figure 4A, and a movie file (Movie 4) is available in the Supporting Information. Initially (frame 60s), the nucleosome has a short left arm and a long right arm. In the next image (frame 62s), the left arm is longer than the right arm, but the situation reverses to the original position after 3 s (frame 65s). This data set shows that transient sliding can occur. The length measurement data are assembled in Figure 4B, demonstrating that this rather stable nucleosome undergoes a fast transient translocation at 62 s. At this time, one arm increases in length accompanied by a decrease in length of another arm by the same value. The process is then fully reversed, resulting in the arms adopting their initial lengths. Note that the size of the nucleosome does not change. These data suggest that the nucleosome can undergo sliding that is transient and reversible. The sliding of the histone core along the DNA molecule occurs at a distance not exceeding 30 nm and takes 3 s.

Dynamics of the Nucleosome in the Presence of CHAPS. We have shown recently that incubation of assembled nucleosomes in the presence of the detergent CHAPS resulted in the formation of nucleosomes with a histone core position not corresponding to the location of the specific 601 sequence.[40] Such nonspecific nucleosomes do not differ from the specific ones by the size of the nucleosome particles or the length of DNA wrapped around the histone core. We hypothesized that the nonspecific nucleosomes were formed from the specific ones by a sliding mechanism.[40] We tested this hypothesis in this analysis by performing time-lapse imaging of nucleosome dynamics in the presence of CHAPS.

AFM images of a set of time-lapse frames are shown in Figure 5A–E. Panel A shows the unprocessed raw image in

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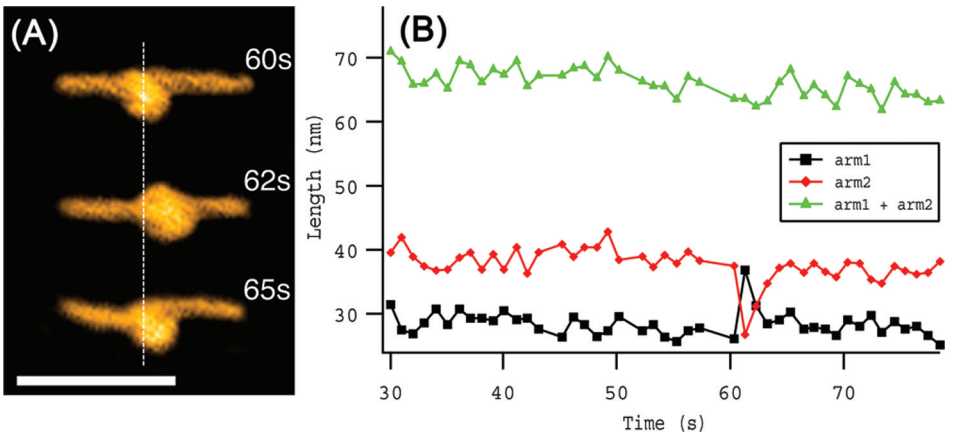

Figure 4. Reversible sliding of the nucleosome. (A) Selected AFM images illustrating a reversible sliding. The vertical dashed line corresponds to the center of nucleosomal particle at 60 s. The numerals (60s, 62s, and 65s) correspond to the times the images were captured. (B) Dependence of the arms' lengths (arm 1, black squares, and arm 2, red diamonds) and the total arms' lengths (green triangles) on time. The scan rate is 1 frame per second. The scale bar in panel A is 50 nm.

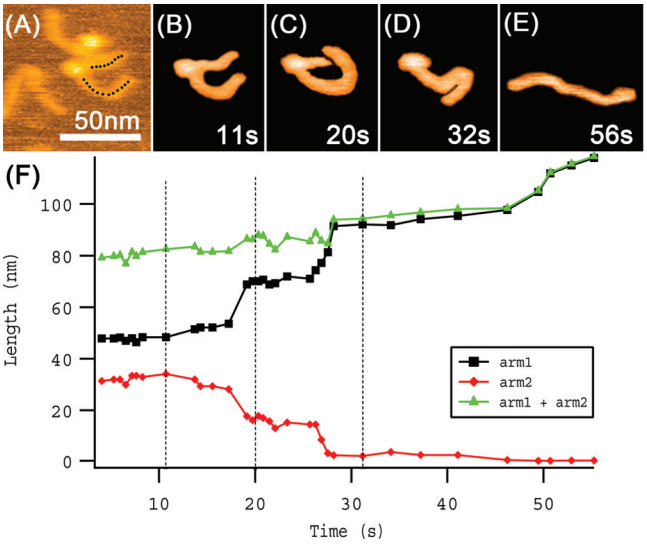

Figure 5. Irreversible sliding of the nucleosome in the presence of CHAPS. (A–E) Set of HS-AFM images. In panel A, the black dotted lines indicate the DNA arms of interest. Panels B–E show the traced images of the molecule on a black background. (F) DNA arm length measurements. Black squares and red diamonds correspond to the lengths of the longer and shorter arms, respectively, in the first image. The green triangles show the time dependence of the total DNA length. The data were acquired with thea scan rate of 1.7 frames per second. The dashed lines in panel F correspond to the times at which panels B–D were acquired (from left to right, respectively).

which the selected nucleosome is indicated with black dotted lines. The nucleosome was traced and placed on a black background to produce panel B. Panels C–E that correspond to images at later times indicated in the figures were obtained the same way. According to the length measurements, the image in panel B corresponds to the position of the nucleosome core at 601 sequences; we termed this a specific complex. Shortening of the small arm is clearly seen in panel C (20 s), and the nucleosome moves to the end of the DNA substrate in panel D (frame 32s). Note that visually, the size of the nucleosome remains unchanged during this repositioning event. The size of the nucleosome decreases during further observation, and the nucleosome dissociates completely in panel E (frame 56s). Length measurements of the DNA arms were performed, and the corresponding graphs are shown in Figure 5F. The changes in length are detected starting at ~15 s, in which a gradual decrease in the length of arm 2 is accompanied with a corresponding increase in the length of arm 1. The first sharp change in the lengths was observed at ~17 s. During the period of one frame, arm 1 increased in length by ~15 nm and arm 2 decreased in length by the same value. There were no substantial changes in lengths until 26 s, when the second stepwise change of arms occurred. At this step, the long arm 1 increases in length by ~20 nm (black squares), accompanied by a decrease in the length of the short arm 2 by half of this value, ~10 nm (red circles). Given the fact that one DNA turn in the nucleosome corresponds to 84 bp or ~25 nm, this stepwise translocation of the nucleosome to the end is accompanied by the nucleosome losing approximately three-fourths of a turn due to unwrapping of long arm 1. This unwrapping process is partially compensated by rewrapping of the short arm, bringing back approximately one-third of the nucleosome turn. After this period, the length of the long arm 1 increases gradually, leading to a complete unwrapping of the nucleosome and the dissociation of the core at 55 s.

Figure 6 illustrates the unwrapping process of the nonspecific nucleosome. See Movie 5 in the Supporting Information for the full set of images. In the subset of images (Figure 6A), the first frame (60 s after start) corresponds to the nucleosome with approximately two nucleosomal turns. The next image (103 s) shows the same nucleosome after unwrapping of approximately one turn. Note that the short arm remains unchanged; therefore, unwrapping is associated with only elongation of the long arm. The unwrapping is almost complete at 167 s, and the core dissociates at 177 s. The length measurement plot is shown in Figure 6B, and a plot of time versus the number of DNA turns calculated from the arms' lengths is shown in Figure 6C. The vertical dashed lines in panels B and C correspond to times at which images shown in Figure 6A were taken. A gradual initial unwrapping process occurs during the first 50 s, followed by the first step at ~70 s during which ~0.4 nucleosome turn is lost. The largest unwrapping step occurs at ~100 s, accompanied by unwrapping of almost one nucleosome turn. The third step (~140 s) leads to a complete unwrapping of the nucleosome.

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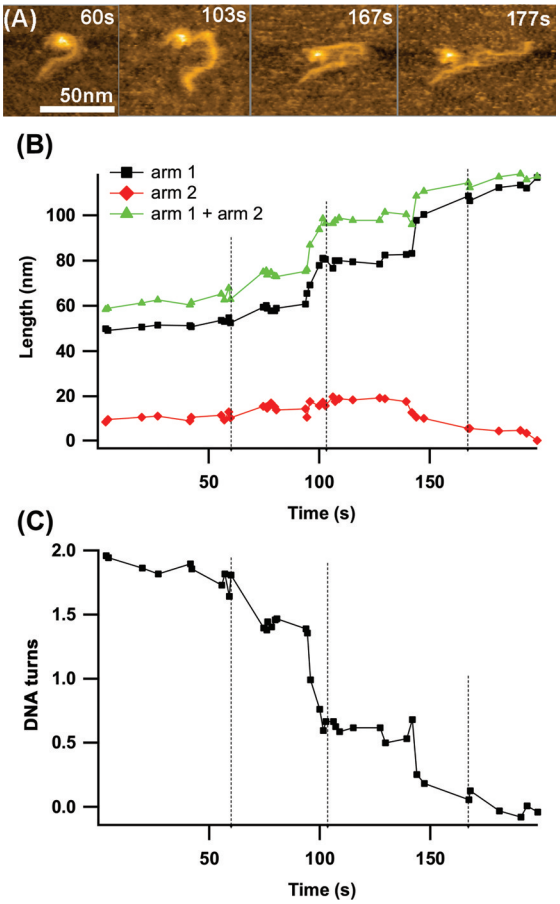

Figure 6. Unwrapping of the nonspecific nucleosome. (A) Selected frames corresponding to a few stages of the unwrapping process. Times are shown in each frame. (B) Time dependence of the lengths of the arms (squares and diamonds) and the total DNA length (triangles) measured for each frame. (C) Time dependence of the number of DNA turns in the nucleosome calculated from the arms' lengths. The data were obtained with a scan rate of 1.7 frames per second. In panels B and C, the dashed lines correspond to the times of first three images shown in A (from left to right, respectively).

DISCUSSION

Nucleosome Unwrapping at High Speed. Previously, we published the finding that nucleosomes are capable of unwrapping spontaneously, as observed by AFM.[26,36] In the time-lapse AFM experiments, nucleosomes underwent a stepwise unwrapping process in which each step could be observed between two consecutive frames. The time resolution under those conditions was ~1 min, implying that either the observed dynamics was that slow or the kinetics on the 1 min time scale were due to the low temporal resolution of the instrument. High-speed AFM revealed that the unwrapping process is fast and complex; it is accompanied by a number of small fast steps spanning ≤1 s. The experiments with subsecond temporal resolution demonstrated that unwrapping of a segment as large as 10 nm (~30 bp) can occur in one ~300 ms step (Figure 2B). The time-resolved fluorescence data of Li et al.[19] showed that the breathing dynamics of nucleosomes occurs on the 50−250 ms time scale, but these studies were not able to measure the spatial range of these fluctuations. Our HS-AFM data show that DNA segments as large as 30 bp dissociate on the 300 ms time scale. Importantly, the images in Figure 3 are of high spatial resolution (the DNA width on the images is ~3 nm). Furthermore, they reveal that the dissociation of an ~30 bp segment (12 s) led to the formation of a loop with a small curvature followed by the relaxation of the loop and repositioning of the entire arm.

Reversible Sliding. The dynamics of nucleosomes is typically described by the site exposure model,[1] according to which DNA dissociates from the histone core by the unwrapping process. Our previous time-lapse AFM data[26] were in line with this model. However, the high-temporal resolution capability of HS-AFM showed that in addition to the site exposure pathway, the sliding pathway is possible. The data in Figure 4 graphically illustrate this capability. The fully wrapped nucleosome (approximately two nucleosomal turns) rolls quickly (~1 s) in one direction over ~38 bp (11 nm), after which it returns to the original position in the same amount of time, without any change in the number of nucleosomal turns, the size of the nucleosome or the angle between the arms. Thus, nucleosomes do slide, although nucleosome dynamics via the exposure model is the predominant pathway.

Dynamics of Nonspecific Nucleosomes and Sequence Specificity. The majority of physical, chemical, and structural studies of nucleosomes were performed with the DNA substrate containing 601 sequence characterized by the highest specificity for nucleosome formation.[32] Indeed, using the vast majority of our current and previous data,[26,36] the nucleosomes are assembled at the 601 sequence leading to nucleosomal particles with lengths of arms corresponding to the arm lengths in Figure S1 of the Supporting Information. We have shown recently that such high sequence specificity can be changed by the incubation of already assembled nucleosomes in a solution containing the zwitterionic detergent, CHAPS.[40] As a result, nucleosome particles with alternative positions relative to the 601 sequence are formed; we termed them nonspecific nucleosomes. Such nucleosomal particles differ from specific ones only by the position of the nucleosome but have the same mean number of nucleosomal turns as specific particles. We suggested previously[40] that nonspecific nucleosomes are formed from specific ones by sliding, and the data in this paper confirm this hypothesis. According to Figure 5, the nucleosome slides by rolling over the short arm. The process primarily occurs in a stepwise fashion, in which unwrapping of the long arm is accompanied by overwrapping of the short arm. The translocation occurs in the range of a second. In addition to the two-step process illustrated in Figure 5, sliding can occur in one step, demonstrated also by the set of data in Figure S2 of the Supporting Information.

Interestingly, sliding is a one-directional process that primarily occurs by the elongation of the long arm and shortening of the short arm. Note that for the AFM experiments, we used positively charged surface interactions, which are a driving force for unfolding of nucleosomes.[26,36] Therefore, the long arms bind more strongly to the surface than the short arms. Fluctuational unwrapping of the long arm further increases the stability of its interactions with the surface, and wrapping of the short arm compensates for the loss of the nucleosome's free energy. As the short arm becomes smaller, the nucleosome becomes capable of directional rolling (sliding) more efficiently. We did not observe this directionality for the specific nucleosome for which the reversible roll was observed (Figure 4). We explain this observation by the effect of the specific 601 sequence that provides the most thermodynami-

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cally favorable location for the nucleosome. Moreover, in our previous experiment, we demonstrated that unwrapping of nucleosomes with the 601 sequence inside the DNA substrate occurs symmetrically, suggesting that the central part of the 601 sequence plays an important role in the sequence-specific positioning of the nucleosome. Therefore, we hypothesize that the low probability of the sliding pathway observed here, as well as in other studies with the use of the 601 sequence, is due to properties of this sequence favoring one pathway over another.

The directionality of sliding further supports our model of the role of electrostatics in the stability and dynamics of nucleosomes.[26,36,40] According to this model, the strengthened interaction of the DNA arms with a positively charged environment (the positively charged surface in our AFM experiments) decreases the stability of the nucleosome. We hypothesized, therefore, that the surface of chromatin remodeling factors accommodating the nucleosome could be positively charged to facilitate nucleosome unfolding,[26] and a recent study from the Kornberg lab[41] supports this hypothesis. In addition to remodeling factors, chromatin interacts with the nuclear membrane that can also modulate chromatin structure and function. Characterization of these interactions is important for elucidating mechanisms controlling chromatin function. AFM as a topographic technique would be a method of choice for such studies. Indeed, although AFM primarily uses mica as a substrate for the imaging and the properties of mica are different from the properties of intracellular surfaces, appropriate modification of the surface may mimic the surface features in vivo. Our studies use a positively charged APS-mica surface, but methods for preparing surfaces with other characteristics are being developed.

Sequence specificity of nucleosome formation is widely discussed in the literature (e.g., refs 42 and 43 and references cited therein). We have shown recently[40] and confirmed in this paper that the stringency for the sequence specificity of nucleosome positioning can be decreased by adding CHAPS to the solution. Importantly, this detergent does not dissociate the nucleosome. On the contrary, it stabilizes the nucleosomes against dissociation at very low nucleosomal concentrations, facilitating the study of nucleosomes by single-molecule techniques. This suggests that other compounds, in addition to CHAPS, can modulate the sequence-specific pattern of nucleosome positioning. Therefore, the analysis of the sequence specificity of nucleosome positioning should take into account the possibility of changing this interaction via environmental conditions. Although CHAPS is not a physiological compound, its structure is rather similar to the structure of cholesterol. We have shown that water-soluble modification of CHAPS stabilizes nucleosomes, although to a lesser extent compared to unmodified CHAPS.[40]

Given the fact that AFM is a technique that directly probes the sample, the effect of the scanning tip on nucleosome dynamics needs to be taken into consideration. The HS-AFM instrument operates in tapping mode; therefore, we need to consider the effect of two major factors: hitting the sample with an oscillating tip and displacement of the sample during scanning. The energy transferred by the tip to the sample is proportional to the square of the oscillation amplitude. HS-AFM operates with an amplitude of ~1 nm, so the estimates provided in the Supporting Information demonstrate that the overall energy induced by the tip is equivalent to an increase in temperature of only ~9.6 K. Given the fact that this energy is distributed over the entire system, including water molecules in the vicinity of the sample, this effect is weak. Another effect of the tip is the displacement of the sample. Because of the high oscillation frequency, the time during which the tip contacts the sample is ~100 ns; therefore, during this period of time, the sample stage moves in the x-direction by only 0.16 nm. This value is comparable to the size of an atom, so the lateral displacement of the sample by the scanning tip is negligible.

■ ASSOCIATED CONTENT

Supporting Information

Potential effects of the instrument characteristics on the sample dynamics, schematics explaining the design of the DNA substrate, graph of the time-dependent change in the length of the short arm during nucleosome sliding, and movies of animated data sets of time-lapse images. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

AFM, atomic force microscopy; NCP, nucleosome core particle; APS, 1-(3-aminopropyl)silatrane; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

■ REFERENCES

(1) Widom, J. (1997) Chromatin: The nucleosome unwrapped. *Curr. Biol. 7*, R653−R655.

(2) Saha, A., Wittmeyer, J., and Cairns, B. R. (2006) Chromatin remodelling: The industrial revolution of DNA around histones. *Nat. Rev. Mol. Cell Biol. 7*, 437−447.

(3) Li, G., and Widom, J. (2004) Nucleosomes facilitate their own invasion. *Nat. Struct. Mol. Biol. 11*, 763−769.

(4) Lovullo, D., Daniel, D., Yodh, J., Lohr, D., and Woodbury, N. W. (2005) A fluorescence resonance energy transfer-based probe to monitor nucleosome structure. *Anal. Biochem. 341*, 165−172.

(5) Leuba, S. H., Anand, S. P., Harp, J. M., and Khan, S. A. (2008) Expedient placement of two fluorescent dyes for investigating dynamic DNA protein interactions in real time. *Chromosome Res. 16*, 451−467.

(6) Blosser, T. R., Yang, J. G., Stone, M. D., Narlikar, G. J., and Zhuang, X. (2009) Dynamics of nucleosome remodelling by individual ACF complexes. *Nature 462*, 1022−1027.

(7) Gurunathan, K., and Levitus, M. (2009) Single-molecule fluorescence studies of nucleosome dynamics. *Curr. Pharm. Biotechnol. 10*, 559−568.

(8) Koopmans, W. J., Brehm, A., Logie, C., Schmidt, T., and van Noort, J. (2007) Single-pair FRET microscopy reveals mononucleosome dynamics. *J. Fluoresc. 17*, 785−795.

(9) Kelbauskas, L., Chan, N., Bash, R., DeBartolo, P., Sun, J., Woodbury, N., and Lohr, D. (2008) Sequence-dependent variations associated with H2A/H2B depletion of nucleosomes. *Biophys. J. 94*, 147−158.

(10) Koopmans, W. J., Buning, R., Schmidt, T., and van Noort, J. (2009) spFRET using alternating excitation and FCS reveals progressive DNA unwrapping in nucleosomes. *Biophys. J. 97*, 195−204.

(11) Zlatanova, J., and Leuba, S. H. (2003) Chromatin fibers, one-at-a-time. *J. Mol. Biol. 331*, 1−19.

(12) Brower-Toland, B. D., Smith, C. L., Yeh, R. C., Lis, J. T., Peterson, C. L., and Wang, M. D. (2002) Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA. *Proc. Natl. Acad. Sci. U.S.A. 99*, 1960−1965.

(13) Bussiek, M., Toth, K., Brun, N., and Langowski, J. (2005) DNA-loop formation on nucleosomes shown by in situ scanning force microscopy of supercoiled DNA. *J. Mol. Biol. 345*, 695−706.

(14) Poirier, M. G., Bussiek, M., Langowski, J., and Widom, J. (2008) Spontaneous access to DNA target sites in folded chromatin fibers. *J. Mol. Biol. 379*, 772−786.

(15) Bussiek, M., Muller, G., Waldeck, W., Diekmann, S., and Langowski, J. (2007) Organisation of nucleosomal arrays reconstituted with repetitive African green monkey α-satellite DNA as analysed by atomic force microscopy. *Eur. Biophys. J. 37*, 81−93.

(16) Montel, F., Menoni, H., Castelnovo, M., Bednar, J., Dimitrov, S., Angelov, D., and Faivre-Moskalenko, C. (2009) The dynamics of individual nucleosomes controls the chromatin condensation pathway: Direct atomic force microscopy visualization of variant chromatin. *Biophys. J. 97*, 544−553.

(17) Anderson, J. D., Thastrom, A., and Widom, J. (2002) Spontaneous access of proteins to buried nucleosomal DNA target sites occurs via a mechanism that is distinct from nucleosome translocation. *Mol. Cell. Biol. 22*, 7147−7157.

(18) Ahmad, K., and Henikoff, S. (2002) Epigenetic consequences of nucleosome dynamics. *Cell 111*, 281−284.

(19) Li, G., Levitus, M., Bustamante, C., and Widom, J. (2005) Rapid spontaneous accessibility of nucleosomal DNA. *Nat. Struct. Mol. Biol. 12*, 46−53.

(20) Bucceri, A., Kapitza, K., and Thoma, F. (2006) Rapid accessibility of nucleosomal DNA in yeast on a second time scale. *EMBO J. 25*, 3123−3132.

(21) Leuba, S. H., Bustamante, C., Zlatanova, J., and van Holde, K. (1998) Contributions of linker histones and histone H3 to chromatin structure: Scanning force microscopy studies on trypsinized fibers. *Biophys. J. 74*, 2823−2829.

(22) Yodh, J. G., Lyubchenko, Y. L., Shlyakhtenko, L. S., Woodbury, N., and Lohr, D. (1999) Evidence for nonrandom behavior in 208-12 subsaturated nucleosomal array populations analyzed by AFM. *Biochemistry 38*, 15756−15763.

(23) Yodh, J. G., Woodbury, N., Shlyakhtenko, L. S., Lyubchenko, Y. L., and Lohr, D. (2002) Mapping nucleosome locations on the 208-12 by AFM provides clear evidence for cooperativity in array occupation. *Biochemistry 41*, 3565−3574.

(24) Nikova, D. N., Pope, L. H., Bennink, M. L., van Leijenhorst-Groener, K. A., van der Werf, K., and Greve, J. (2004) Unexpected binding motifs for subnucleosomal particles revealed by atomic force microscopy. *Biophys. J. 87*, 4135−4145.

(25) Lyubchenko, Y. L., Shlyakhtenko, L. S., and Gall, A. A. (2009) Atomic force microscopy imaging and probing of DNA, proteins, and protein DNA complexes: Silatrane surface chemistry. *Methods Mol. Biol. 543*, 337−351.

(26) Shlyakhtenko, L. S., Lushnikov, A. Y., and Lyubchenko, Y. L. (2009) Dynamics of nucleosomes revealed by time-lapse atomic force microscopy. *Biochemistry 48*, 7842−7848.

(27) Ando, T., Kodera, N., Takai, E., Maruyama, D., Saito, K., and Toda, A. (2001) A high-speed atomic force microscope for studying biological macromolecules. *Proc. Natl. Acad. Sci. U.S.A. 98*, 12468−12472.

(28) Ando, T., Uchihashi, T., Kodera, N., Yamamoto, D., Taniguchi, M., Miyagi, A., and Yamashita, H. (2007) High-speed atomic force microscopy for observing dynamic biomolecular processes. *J. Mol. Recognit. 20*, 448−458.

(29) Crampton, N., Yokokawa, M., Dryden, D. T., Edwardson, J. M., Rao, D. N., Takeyasu, K., Yoshimura, S. H., and Henderson, R. M. (2007) Fast-scan atomic force microscopy reveals that the type III restriction enzyme EcoP15I is capable of DNA translocation and looping. *Proc. Natl. Acad. Sci. U.S.A. 104*, 12755−12760.

(30) Gilmore, J. L., Suzuki, Y., Tamulaitis, G., Siksnys, V., Takeyasu, K., and Lyubchenko, Y. L. (2009) Single-molecule dynamics of the DNA-EcoRII protein complexes revealed with high-speed atomic force microscopy. *Biochemistry 48*, 10492−10498.

(31) Suzuki, Y., Higuchi, Y., Hizume, K., Yokokawa, M., Yoshimura, S. H., Yoshikawa, K., and Takeyasu, K. (2010) Molecular dynamics of DNA and nucleosomes in solution studied by fast-scanning atomic force microscopy. *Ultramicroscopy 110*, 682−688.

(32) Lowary, P. T., and Widom, J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol. 276*, 19−42.

(33) Filenko, N. A., Kolar, C., West, J. T., Smith, S. A., Hassan, Y. I., Borgstahl, G. E. O., Zempleni, J., and Lyubchenko, Y. L. (2011) The Role of Histone H4 Biotinylation in the Structure of Nucleosomes. *PLoS One 6*, e16299.

(34) Luger, K., Rechsteiner, T. J., Richmond, T. J., Wassarman, Paul M., and A., P. W. (1999) Preparation of nucleosome core particle from recombinant histones. *Methods Enzymol. 304*, 3−19.

(35) Shlyakhtenko, L. S., Gall, A. A., Filonov, A., Cerovac, Z., Lushnikov, A., and Lyubchenko, Y. L. (2003) Silatrane-based surface chemistry for immobilization of DNA, protein-DNA complexes and other biological materials. *Ultramicroscopy 97*, 279−287.

(36) Lyubchenko, Y. L., and Shlyakhtenko, L. S. (2009) AFM for analysis of structure and dynamics of DNA and protein-DNA complexes. *Methods 47*, 206−213.

(37) Lyubchenko, Y. L. (2011) Preparation of DNA and nucleoprotein samples for AFM imaging. *Micron 42*, 196−206.

(38) Lyubchenko, Y. L., Shlyakhtenko, L. S., and Ando, T. (2011) Imaging of nucleic acids with atomic force microscopy. *Methods 54*, 274−283.

(39) Uchihashi, T., and Ando, T. (2011) High-speed atomic force microscopy and biomolecular processes. *Methods Mol. Biol. 736*, 285−300.

(40) Menshikova, I., Menshikov, E., Filenko, N., and Lyubchenko, Y. L. (2011) Nucleosomes structure and dynamics: Effect of CHAPS. *Int. J. Biochem. Mol. Biol. 2*, 129−137.

(41) Lorch, Y., Maier-Davis, B., and Kornberg, R. D. (2010) Mechanism of chromatin remodeling. *Proc. Natl. Acad. Sci. U.S.A. 107*, 3458−3462.

(42) Kaplan, N., Moore, I., Fondufe-Mittendorf, Y., Gossett, A. J., Tillo, D., Field, Y., Hughes, T. R., Lieb, J. D., Widom, J., and Segal, E. (2010) Nucleosome sequence preferences influence in vivo nucleosome organization. *Nat. Struct. Mol. Biol. 17*, 918−920, , author reply 920.

(43) Tillo, D., Kaplan, N., Moore, I. K., Fondufe-Mittendorf, Y., Gossett, A. J., Field, Y., Lieb, J. D., Widom, J., Segal, E., and Hughes, T.

R. (2010) High nucleosome occupancy is encoded at human regulatory sequences. *PLoS One 5*, e9129.