Phase Transition in Reconstituted Chromatin

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Abstract

By observing reconstituted chromatin by fluorescence microscopy (FM) and atomic force microscopy (AFM), we found that the density of nucleosomes exhibits a bimodal profile, i.e., there is a large transition between the dense and dispersed states in reconstituted chromatin. Based on an analysis of the spatial distribution of nucleosome cores, we deduced an effective thermodynamic potential as a function of the nucleosome-nucleosome distance. This enabled us to interpret the folding transition of chromatin in terms of a first-order phase transition. This mechanism for the condensation of chromatin is discussed in terms of its biological significance.

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Genomic DNA in eukaryotes is compactly folded into chromatin through several hierarchical packings [1]. The fundamental unit of such packing, the nucleosome, consists of 146 bp of DNA wrapped around a histone octamer (two molecules each of H2A, H2B, H3 and H4). The semi-flexible DNA chain wraps around a histone core about two turns [2]. It is widely expected that the manner of packing and the dynamics of nucleosomes are associated with gene activities in living cells [3–6]. There have been many studies on the static [7–10] and dynamic [11] properties of nucleosomes. It has been shown that nucleosomes condense under various conditions; for example, under a high salt concentration [12]. However, the nature of the higher-order structure is not well understood and the underlying physics of nucleosome condensation have not yet been clarified. It has been suggested, for example, that measurement of the actual interaction energy between nucleosomes is essential [8,11,13,14] for obtaining deeper insight into chromatin condensation, but there have been no experimental studies on the interaction potential.

On the other hand, it has recently been found that linear DNA larger than several tens of base pairs exhibits a large discrete coil-globule transition, accompanied by a change in density on the order of $10^4$–$10^5$, upon the addition of various kinds of condensing agents [15,16]. It has been revealed that this transition is a first-order phase transition under the criterion of Landau, i.e., an ON/OFF transition from an elongated coil state to a compact globule state [17]. Very recently, such an ON/OFF transition of DNA has been suggested to play an important role in gene activity [17]. Discreteness of the coil-globule transition is a general characteristic of single semi-flexible polymer chains [18].

The purpose of this study was to obtain a deeper understanding of the conformational changes of chromatin, which is essential for obtaining insight into genetic activity including duplication, transcription, etc. We investigated the physical properties of reconstituted chro-
matin using fluorescence microscopy (FM) and atomic force microscopy (AFM). We obtained the pair interaction potential between nucleosome cores from an analysis of AFM images and used it to dissect the mechanism of chromatin compaction, or chromatin condensation.[19]

The preparation of core histones and 106-kbp plasmids (circular DNA) and the reconstitution of chromatin were carried out as previously reported.[20] In this study, the mass ratio [histone]/[DNA] was set to 1.0 and 1.3. Concentration of NaCl, which affects the interaction between nucleosomes[7], was 50 mM throughout this article. Reconstituted chromatin samples were fixed with 0.3% glutaraldehyde in 10mM Hepes-NaOH [pH 7.5] for 30 minutes at 25°C. The chromatin was placed on a freshly cleaved thin piece of mica (thickness; ca. 30–50 µm) stuck to a glass cover plate (Matsunami Glass, No. 1, Japan) for FM observation. The mica surface was pre-treated with 10 mM spermidine. This treatment was performed so that chromatin would adsorb onto the mica surface. The chromatin complexes were visualized by fluorescent microscopy using a fluorescent dye, 0.1 µM 4′, 6-diamidino-2-phenylindole (DAPI). The observation was performed on a droplet (10 µL) instilled on mica. The sample droplet on mica was washed with Millipore water and blown dry with nitrogen gas for 5 minutes. Fluorescent chromatin complexes were observed under a Zeiss Axiovert 200 microscope with a 100× oil-immersed objective lens at 25°C, and recorded on Axio Vision with an AxioCam camera. To obtain two-dimensional real-time fluorescent image data, an inverted microscope (IX-70, Olympus) with a 100× oil-immersed objective lens and a highly sensitive EB-CCD camera with an image-processing system (Hamamatsu Photonics) were used. The video data were recorded on videotapes, and then analyzed with personal computers. Due to the blurring effect[21] in the observation with a highly sensitive video system, the size of observed DNA images was assumed to be slightly larger (ca. 0.3
µm) than the actual size of the chromatin. The reconstituted chromatin structures observed by FM observation were analyzed under Tapping Mode™ in AFM (Nanoscope Bioscope, Digital Instruments) in air at room temperature. Both FM and AFM images were obtained on exactly the same chromatin. The distance distribution of nucleosomes was obtained on AFM images acquired with a high-resolution AFM apparatus (Nanoscope IIIa).

Figure 1 shows quasi-three-dimensional (3D) FM images (A–C), corresponding 2D images (insets), and AFM images (D–F) of reconstituted chromatin adsorbed onto a mica surface. The pictures in Fig. 1 (A, D), (B, E), and (C, F) show exactly the same molecules [22]. The mass ratio [histone]/[DNA] is 1.0 in (A, D) and 1.3 in (B, C, E, and F). In (D), nucleosomes are dispersed in the chromatin, whereas in (E) the condensed and dispersed parts coexist. In (F) the chromatin is entirely condensed. Under the condition [histone]/[DNA] = 1.3, the partially and entirely condensed states exist in almost equal proportions, while there is a very low proportion (<5%) of the dispersed state. Under [histone]/[DNA] = 1.0, more than 95% of chromatin is in the dispersed state, while the remainder is in the partially condensed state. Despite the low resolution, FM observation provides information on the degree of condensation of individual reconstituted chromatin in the bulk solution as well as on the surface.

To evaluate the actual size of reconstituted chromatin in bulk solution, we measured the Brownian motion of individual chromatin complexes using FM. From the time-dependence of the mean square displacement of the center of mass of chromatin, we obtained the diffusion constant \( D \) using the following relationship: \( \langle (\mathbf{r}(0) - \mathbf{r}(t))^2 \rangle = 4Dt \) [23]. The hydrodynamic radius \( R_H \) is thus deduced as in Table I using the Stokes-Einstein relationship, \( R_H = k_B T / (6\pi \eta D) \), where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature and \( \eta \) is the viscosity of the solvent (0.89 mPas for pure water at \( T = 297 \) K). We also
evaluated the size of reconstituted chromatin by AFM measurement. The major axis $R_L$ and the minor axis $R_S$ of reconstituted chromatin were measured in two-dimensional AFM images. Table I shows $R_L$ and $R_S$, together with $R_{AFM} = \sqrt{R_L R_S}$. The relative ratio of $R_{AFM}/R_H$ is almost the same for [histone]/[DNA] ratios both of 1.0 and 1.3, regardless of the difference in the observed state, i.e., the former is chromatin on a surface and the latter is chromatin in bulk solution.

We obtained the distribution of distances $N(r)$ as a function of the distance $r$ between pairs of nucleosomes in AFM images. It is expected that nucleosomes exhibit a nearly equilibrium structure on a 2D mica surface [24]. We assumed that the radial distribution function $g(r)$ is proportional to $N(r)/r$. Since the nucleosome density is low enough with [histone]/[DNA] = 1.0 (see Fig. 1(A)), the pair potential can be roughly deduced from $g(r)$ assuming a Boltzmann distribution, $U(r) = -k_B T \ln g(r)$ [25–27], where $g(r)$ and $U(r)$ were calculated after fitting $N(r)$ to a polynomial. The analysis was performed for [histone]/[DNA] = 1.0, since in this condition we can count all of the nucleosomes in reconstituted chromatin. The result is shown in Fig. 2 and indicates that $U(r)$ has a minimum 0.3 $k_B T$ depth at $r \approx 13$ nm. By fitting the experimental data in Fig. 2 with the following equation of potential, $U(r) = -A r^{-\mu} + B r^{-\nu}$ ($\mu$, $\nu$: integer; $\mu < \nu$), we obtain $A = 2.4$, $B = 12$, $\mu = 9$, and $\nu = 23$, where $r$ is normalized with the diameter of a nucleosome (11 nm). These values are much larger than those of the Lennard-Jones potential ($\mu = 6$ and $\nu = 12$). The large values of the exponents can be attributed to the large excluded volume of the nucleosome core. A similar profile of potential, i.e., relatively large values for the exponents $\mu$ and $\nu$, can also be deduced from an analysis of the interaction of neighboring nucleosomes along the DNA chain (data not shown). Such large values of the exponents can explain the discrete nature of the transition as described below.
Next, we will discuss the conformational stability of chromatin based on the above pair interaction energy. The total free energy $F$ of reconstituted chromatin with $n$ nucleosomes can be described as

$$F(n) = F_{\text{ela}} + F_{\text{int}}(n),$$

(1)

where $F_{\text{ela}}$ is the entropic elasticity of the DNA chain and $F_{\text{int}}(n)$ is the volume interaction between nucleosomes. We neglect the volume interaction of double-stranded DNA, since the thickness of DNA ($\sim 2$ nm) is much smaller than the diameter of a nucleosome ($\sim 11$ nm).

Using the swelling parameter $\alpha$, we obtain

$$F_{\text{ela}}/k_B T = \alpha^2 + \alpha^{-2},$$

(2)

where $\alpha^2 = \langle R^2 \rangle / \langle R_0^2 \rangle$. $\langle R^2 \rangle$ is the mean square of the radius of gyration and $R_0$ is the analogous size of an ideal Gaussian coil. $\alpha^2$ and $\alpha^{-2}$ correspond to extension and compression of the chain, respectively [28]. Assuming that $U(r)$ has a narrow minimum so that nucleosome-nucleosome interaction occurs only among the nearest neighbors, we can take $F_{\text{int}}(n) \sim n U(r)$. By adapting $U(r) = -Ar^{-9} + Br^{-23}$, $F_{\text{int}}(n)$ can be written as

$$F_{\text{int}}(n, \rho)/k_B T \sim -An\rho^3 + Bn\rho^{7.7},$$

(3)

where $\rho$ is the normalized density of nucleosomes, $\rho \approx r^{-3}$. Thus, we obtain

$$F(n, \rho)/k_B T \sim \alpha^2 + \alpha^{-2} - An\rho^3 + Bn\rho^{7.7}.$$ 

(4)

In the present model, $R_0$ decreases with an increase in $n$. Thus, we define $L$ as the apparent
contour length of chromatin, \( L = L_0 - an \), where \( L_0 \) is the contour length of DNA without any histones (36 \( \mu \)m for this sample), and \( a \) is the length of DNA wrapped around a histone octamer, ca. 50 nm (146 bp). With the Kuhn length \( \lambda \) (100 nm for a DNA chain) and the number of Kuhn segments \( N_S \), the size of an ideal Gaussian chain is described as \( \langle R_0^2 \rangle^{1/2} = \lambda N_S^{1/2} \). Since \( N_S = L/\lambda \), we obtain a modified \( R_0 \), which we call \( R'_0 \), \( \langle R'_0^2 \rangle^{1/2} = \lambda (L/\lambda)^{1/2} = \lambda^{1/2}L^{1/2} \). Using the relation \( \rho \sim nR^{-3} \), we obtain

\[
\frac{F(n, \rho)}{nk_BT} = \frac{\rho^{-2/3}}{n^{1/3} \lambda (L_0 - an)} + \frac{\lambda (L_0 - an)}{n^{5/3}} \rho^{2/3} - A\rho^3 + B\rho^{7/7}.
\]

(5)

It is obvious that \( F(n, \rho)/nk_BT \) has two minima. The one at the lower-density region is derived from the first and second terms in Eq. (5) and the other at the higher-density region is derived from the third and fourth terms. As \( n \) increases, the minimum of the condensed state becomes deeper while a double-minimum profile is maintained. When the two minima have a similar depth, two different states of high and low nucleosome density coexist. Figure 3 shows the free-energy profiles of nucleosomes with \( n = 400, 500 \) and 600 calculated with Eq. (5) together with schemes of the corresponding conformations in three dimensions. The observed result regarding the elongated conformation in Fig. 1 corresponds to the deeper minimum at \( \rho \approx 0 \). With an increase in \( n \), the free energy of the condensed state becomes the absolute minimum, which reproduces the experimental trend. In the actual experiment for \([\text{histone}]/[\text{DNA}] = 1.3\), there are two different states: fully condensed and partially condensed. Any instability due to the interfacial energy between the condensed and dispersed parts should be negligible considering the zero-dimensional nature of the interface. Therefore, we can expect the appearance of an intrachain phase-segregated state as in Fig. 1(B). Previous studies on native linear DNA chains have indicated that a phase-segregated
state is actually observed in individual DNA molecules and that the characteristic scale of segregation depends on the degree of the surviving electronic charge in the condensed part [29,30].

Several biological studies have demonstrated that the higher-order folding of chromatin fiber and its dynamic structural changes largely depend on the proper functions of various structural and regulatory proteins in the nucleus [31,32], which are critical for gene expression and chromosome segregation. On the other hand, the results obtained in this study, together with those in several previous studies [11,33], suggest that the physical properties of a DNA strand (length and superhelicity) and the interaction between nucleosomes play fundamental roles in chromatin dynamics. The higher-order architecture of chromatin is determined by the fundamental properties of chromatin fiber itself. In this sense, it should be noted that chromosomes are composed of several chromatin loops on the order of \(\sim 100\) kb [34–36], which is approximately the same length as used in this study. A first-order large-scale conformational transition may explain why previous experiments have failed to observe an intermediate state in chromatin condensation. More importantly, it may explain the switching of a large number of genes. Recently, it has been reported that transcription is completely inhibited through an all-or-none transition in the structure of giant DNA molecules [17,37]. Thus, we would like to propose a hypothesis of gene self-regulation through a combination of large-scale ON/OFF switching due to a conformational transition and several regulatory factors to bind specific regions.

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In this paper, we use the term ‘condensation’ to describe the apparent aggregation of nucleosomes in vitro. In general, the term ‘aggregation’ means random concentration, while ‘condensation’ or ‘compaction’ has biological significance and reflects ordered packing such as in chromatin condensation. The structures we observed are likely to be biologically significant because they mimic the situation in vivo. Therefore, we would like to use the term ‘condensation’.

We performed AFM measurements on reconstituted chromatin with and without DAPI, and confirmed that there is essentially no difference between these conditions.

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TABLE I: The major axis of a chromatin $R_L$, the minor axis $R_S$, $R_{AFM}$, and the hydrodynamic radius $R_H$. $R_L$ and $R_S$ were measured in AFM images by assuming that a chromatin complex is elliptical. $R_H$ was measured in fluorescent images. $R_{AFM}$ ($=\sqrt{R_L R_S}$) and $R_H$ are the hypothetical radii of chromatin if they are considered to be spheres on a mica surface and in bulk solution, respectively.

| [histone]/[DNA] | $R_L$ (nm) | $R_S$ (nm) | $R_{AFM}$ (nm) | $R_H$ (nm) |
|-----------------|------------|------------|----------------|------------|
| 1.0             | 420 ± 180  | 250 ± 90   | 320 ± 120      | 370 ± 70   |
| 1.3             | 220 ± 100  | 130 ± 90   | 170 ± 100      | 190 ± 50   |
Figure Captions

FIG. 1: Reconstituted chromatin with [histone]/[DNA] = 1 (A, D) and 1.3 (B, C, E, and F). The images in (A, D), (B, E), and (C, F) are photographs of the same chromatin complex. (A–C) Light-intensity distribution of fluorescent images of reconstituted chromatin situated on a mica surface. Insets are corresponding 2D fluorescent images. (D–F) AFM images of the same chromatin as in the fluorescent images. The scale bar is 0.2 µm in AFM images.

FIG. 2: The pair potential of nucleosomes obtained by an equation for the Boltzmann distribution. Inset: The nucleosome-nucleosome distance distribution $N(r)$ of chromatin reconstituted with [histone]/[DNA] = 1.0. $U(r)$ is deduced by polynomial curve-fitting of $N(r)$.

FIG. 3: Free-energy profiles of a nucleosome with $n = 400$, 500, and 600 and corresponding schematic representations, where $n$ is the number of nucleosomes in a single chromatin complex. The free energy as a function of the normalized density of nucleosomes in the three-dimensional conformation was calculated from Eq. (5).
