Thermodynamics of Nucleosomal Core Particles†

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ABSTRACT: In this paper, a numerically detailed thermodynamic investigation of nucleosomal core particles is presented. The nonlinear Poisson−Boltzmann equation governs the electrostatic properties of both the DNA and histone protein. Brownian dynamics is used as the leading method, in combination with the analysis of the electrical features of the nucleosome. At elevated temperature, the structure of the nucleosome is destabilized by the decrease in electrical interactions of DNA−histone complexes, which can be explained with the EDL theory. Two obvious unwrapping transitions can be found, occurring within the temperature ranges 43−52 and 65−80 °C. The first transition is characterized by the melting of DNA terminal domains, and the feature of the second transition is the massive unwrapping of the DNA middle domain. It can be concluded that the nucleosomal DNA consists of two distinct structures, where the DNA terminal domains are less tightly bound to the histone than the DNA middle domain.

DNA in the eukaryotic nucleus is ordered into chromatin through several hierarchical condensations, and the repeating structural unit is called a nucleosome. The nucleosomal core particle consists of 146 bp of DNA wrapped in 1.75 turns of a left-handed helix around an octamer (two copies of each of H2A, H2B, H3, and H4) (*1*−*4*). Important biological functions of chromatin, such as replication and transcription, are highly regulated by the changes of nucleosome structure and conformation. It is well-known that the conformational changes in the nucleosome are sensitive to salt concentration, pH, and temperature of local environment. Temperature effects are expected to play an important role in the stability of the nucleosome.

Several research groups have attempted to experimentally study the thermodynamics of the nucleosome (*5*−*11*). Thermal denaturation of very homogeneous preparations of core particles has been studied by Weischet et al. (*12*). The melting of core particles is interpreted as a biphasic denaturation of nucleosomal DNA. On the basis of this model, McGhee and Felsenfeld have analyzed the salt dependence of the melting temperature of the first transition and estimated that only 15% of the phosphates of the DNA termini are involved in intimate charge−charge interactions with histone. The fact that the wrapping of the DNA around the histone octamer involves a large energy change has also been demonstrated by the analysis of thermodynamics of condensation of nuclear chromatin (*13*). Recent investigations have focused on the effect of the histone tail on the thermodynamic behavior of the core histone complex (*14*). These experimental studies have provided some insights into some temperature-induced structural changes of nucleosomes. But some disagreement in conclusions results from the variation of prepared nucleosomal samples used in different experiments.

There are also many numerical studies on the stability of the nucleosome. Kunze and Netz explored the DNA−histone complex in low, intermediate, and high concentrations, characterized by rotational and mirror symmetries (*15*). In the following research, they emphasized the effects of salt concentration, DNA length variation, and DNA charge renormalization on the nucleosomal structures (*16*). Using Brownian dynamics, the effect of the DNA chain end on the positioning of the histone core particle has been reported (*17*). These simple numerical schemes are very useful for compensating the experimental study of the characteristics of the nucleosome. Unfortunately, these studies emphasize on the salt-induced nucleosomal structure but not directly on the thermodynamics of the nucleosome. Moreover, they described the electrostatic interaction between charges on the DNA with each other and the histone by Debye−Hückel potential, without considering nonlinear effects.

In this paper, we use the full nonlinear Poisson−Boltzmann theory to govern the electrostatic properties of both DNA and histone. We attempt to shed further light on the mechanism of temperature-induced conformational change in nucleosomal core particles. The paper is organized as follows. First, we present the numerical model describing the DNA chain and histone core particle, emphasizing on the Poisson−Boltzmann theory. Next, we discuss the unwrapping transitions induced by raising the temperature using Brownian dynamics. The last section presents some concluding remarks and raises some possible issues.

MODEL

Here, we feel it worthwhile to provide some background on the DNA and histone core particle surrounded by the solution. This should serve as an aid in understanding the change in nucleosomal conformation.

DNA is a negatively charged macromolecule in contact with an electrolyte due to phosphate groups on the helix

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backbone. The electrostatic charges will attract the counterions in the liquid and influence the distribution of nearby ions in the electrolyte solution. Then an electric field is established, where the counterion-rich region is referred to as the electrical double layer (EDL). It is also known that the histone octamer is shaped as a disk of diameter 11 nm and thickness 8 nm, containing many positively charged residues. Similarly, negatively charged counterions can be attracted, and an electrical double layer is formed around the histone octamer.

The property of the electrical double layer is governed by the nonlinear Poisson−Boltzmann equation, which relates the electrostatic potential and ion concentration distribution:

$$\nabla^2\Phi = k^2 \sinh \Phi \tag{1}$$

where Φ is the dimensionless electrostatic potential at any point, $\Phi = ze\Psi/k_BT$, where Ψ is the scalar electrostatic potential, k_B is the Boltzmann constant, T is the temperature, z is the valence of ions, and e is the charge of an electron. By defining the parameter:

$$k^2 = 2z^2e^2n_0/\epsilon k_BT \tag{2}$$

where n_0 is the bulk concentration and ϵ is the dielectric constant of the solution. $1/k$ is normally referred to as the EDL thickness.

If the dimensionless surface potential is Φ^0, eq 1 is subjected to the following boundary conditions:

$$\Phi|_{s=0} = \Phi^0 \tag{3}$$

where s is the distance away from the surface, and

$$\Phi|_{s\to+\infty} = 0 \tag{4}$$

To our knowledge, the full nonlinear form of the Poisson−Boltzmann equation has no analytical solution in a finite fluid. However, if it is assumed that $\Phi = ze\Psi/k_BT \leq 1$, solution is a simple exponential decay in the Debye−Hückel limit:

$$\Phi = \Phi^0 \exp(-ks) \tag{5}$$

Due to nonlinear effects, the Debye−Hückel approximation is not valid to predict the distribution of potential at low salt concentration, low temperature, and high temperature. In this paper, we use the finite difference method to numerically solve the nonlinear Poisson−Boltzmann equation. The nonlinear source term, sinh Φ, can be linearized as

$$\sinh \Phi_{n+1} = \sinh \Phi_n + (\Phi_{n+1} - \Phi_n)\cosh \Phi_n \tag{6}$$

where Φ_{n+1} and Φ_n denote the $(n+1)$th and nth iterative potential, respectively. The derived discrete, algebraic equations are solved by using the Gauss−Seidel iterative procedure, with the successive overrelaxation technique employed to improve the convergence time (*18*). Electrical potential, Φ, sharply decays near the surface of the sphere and slowly decreases far from the surface; the variable grid spacing is employed to reduce the truncation error (*19*).

According to the above numerical scheme, the electrical potential distribution $\Phi(s)$ has been obtained. In the DNA−histone complex, the histone protein is modeled as a uniformly charged, rigid sphere with the charge Ze over its surface (e is the elementary charge). According to Verwey's and Overbeek's relation between the surface potential and the surface charge Ze (*20*), we know

$$\Phi_h^0 = \frac{Z_he}{4\pi\epsilon R}\left(\frac{1}{1+kR}\right) \tag{7}$$

where Φ_h^0 and Z_he are the surface potential and the surface charge of the histone, respectively. R is the radius of the histone, $R = 4nm$. Solving eq 1 with the boundary conditions (eqs 4 and 7), the electrostatic potential of histone protein, $\Phi_h(s)$, can be obtained. DNA is negatively charged and histone protein is positively charged, so the attraction potential of each DNA monomer exerted by the histone protein has been found:

$$E_A = \Phi_h(s) \tag{8}$$

where s is the distance between the histone and DNA monomer. DNA is modeled as N beads connected by $N-1$ bonds. If the full surface charge of the DNA chain is Z_De, the surface potential of each monomer can be expressed:

$$\Phi_D^0 = \frac{Z_De}{N4\pi\epsilon r_D}\left(\frac{1}{1+kr_D}\right) \tag{9}$$

where r_D is the radius of DNA monomer. Solving eq 1 with the boundary conditions (eqs 4 and 9), the electrostatic potential of each DNA monomer, $\Phi_D^j(s)$, $j = 1, 2, ..., N$, can be obtained. Each DNA monomer is negatively charged, so the repulsion potential of the ith DNA monomer has been found:

$$E_R = \sum_{j=1,i\neq j}^{N} \Phi_D^{ij}(s) \tag{10}$$

where i, j are the indices of the DNA monomer, s is the distance between the ith monomer and the jth monomer, and Φ_D^{ij} is the repulsion potential of the ith DNA monomer exerted by the jth DNA monomer.

To model the DNA stiffness, the following bending potential is introduced:

$$E_B = g\sum k_BT\left[1 - \frac{(r_{i-1} - r_i)(r_i - r_{i+1})}{(2r_D)^2}\right] \tag{11}$$

where g is the dimensionless force constant for bending and r is the monomer position vector for complete description of a conformation of the system.

The complex potential energy is assumed to be

$$E = E_A + E_R + E_B \tag{12}$$

The generalized Langevin equation of translational motion can be expressed as (*21*, *22*)

$$m\dot{v}_i = -\sum_{j=1}^{N}\zeta_{i,j}v_i + F_i + \sum_{j=1}^{N}\alpha_{i,j}f_j \tag{13}$$

where v_i is the velocity of the ith bead and F_i is the sum of

interparticle forces acting on bead i and can be obtained from the usual relation: $F_i = -\nabla_i E$. Thus $\sum_j \alpha_{i,j} f_j$ represents the randomly fluctuating force exerted on a DNA monomer or the histone protein by the surrounding fluid. The f_j are described by a Gaussian distribution with the mean and covariance

$$\langle f_j \rangle = 0 \tag{14}$$

$$\langle f_i(t) f_j(t') \rangle = 2\delta_{i,j}\delta(t - t') \tag{15}$$

The configuration-dependent friction tensor ζ_{ij} can be calculated according to Stokes' law. The coefficients α_{ij} are related to the hydrodynamic friction tensor by

$$\zeta_{i,j} = \frac{1}{k_B T}\sum_l \alpha_{i,l}\alpha_{l,j}^T \tag{16}$$

and the coefficients α_{ij} are given by

$$\alpha_{ii} = (\zeta_{ii} - \sum_{k=1}^{i-1}\alpha_{ik})^{1/2} \tag{17}$$

$$\alpha_{ij} = (\zeta_{ij} - \sum_{k=1}^{j-1}\alpha_{ik}\alpha_{jk})/\alpha_{jj} \qquad (i > j) \tag{18}$$

RESULTS AND DISCUSSION

The total number of basic amino acids in the histone protein is 216 (*23*), but it is not clear whether all basic amino acids are dissociated at such high temperature used in this paper. So we fix $Z_h = 50$ to explore the thermodynamics of the nucleosome. Considering the DNA with 146 base pairs of length 0.34 nm each, the total charge on the DNA is $Z_D = 292$ and is uniform distribution. The bending constant g can be used to regulate the stiffness of the DNA chain, $g = 8$. We retain the salt concentration of 0.1 M.

Before solving the generalized Largevin equation, it should be pointed out that the problem we are dealing with is extremely complicated. It can be found that a change in temperature may have impact on the EDL thickness and hence the entire EDL field. Such influences can be reflected from the parameter defined as $k^2 = 2z^2e^2n_0/\epsilon k_B T$. In other words, the increase in temperature may cause alterations in distributions of both attraction potential E_A and repulsion potential E_R. However, it must be noted that the other parameters cannot stay invariant with the alteration of temperature. Particularly, the dielectric constant of the solution, ϵ, alternates oppositely to the temperature variation. It just means that the increase in temperature may lead to the decrease in the dielectric constant. The last but the most important is that the activity of counterions in the EDL will enhance due to the added temperature, which leads to the larger EDL thickness. So a dimensionless parameter s must be introduced to revise the parameter $k^{*2} = s^*(2z^2e^2n_0/\epsilon k_B T)$ (*12*, *24*).

In Figure 1, the distribution of the modified parameter k^* vs temperature T (solid line) is plotted compared with the initial parameter k vs temperature T (dashed line). As seen from Figure 1, the modified parameter k^* exhibits significantly stronger dependence on the increase in temperature than the initial parameter k.

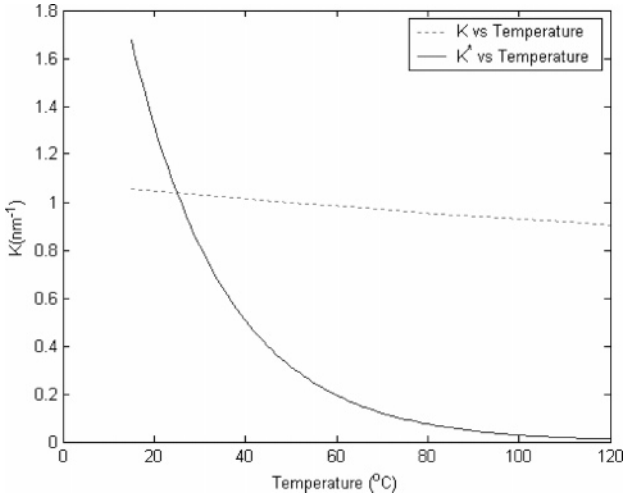

FIGURE 1: Comparison of distribution for modified Debye−Hückel parameter K^* and initial parameter K vs temperature.

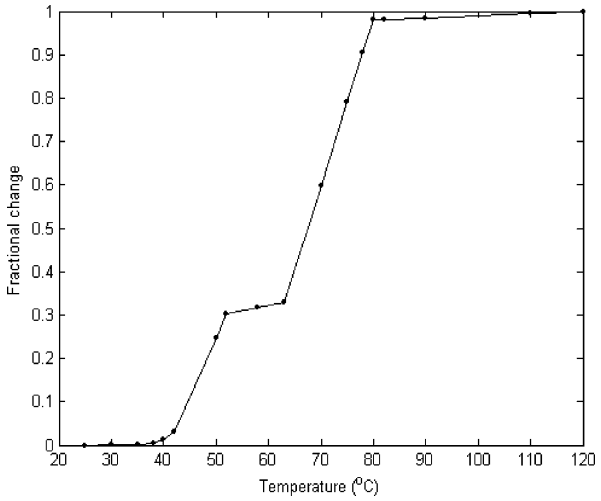

FIGURE 2: Analysis of thermal dynamics of the nucleosomal core particle. The conformational transition is biphasic. The first transition is at 43−52 °C; the second transition is at 65−80 °C.

In this paper, the thermodynamics of nucleosomal core particles refers to analysis of alteration in the shape of the histone−DNA complex at varying temperatures, using Brownian dynamics. Figure 2 shows the results of the analysis over a temperature range of 25−120 °C. It can be found that the number of the DNA monomers that wrapped the histone protein decreases sharply between 43 and 52 °C and then smoothes away until 63 °C. The massive unwrapping of DNA segments from the histone protein begins at the temperature of 65 °C and finishes the complete unwrapping behavior at about 80 °C, where a nucleosome is separated into a free DNA chain and the histone protein.

In the initial stage (25−30 °C), the nucleosome exhibits a compact form where 146 base pairs of DNA wrapped tightly in 1.75 turns around the histone protein as shown in Figure 3. With the elevated temperature, the conformational transition occurs at the temperature of 43 °C for unwrapping up to 20 base pairs of DNA at each end of the chain from the histone core, which is caused by the stronger repulsion potential of DNA strand competition with attraction potential of histone−DNA. So the second stage presents the stretched form where about 100 base pairs of DNA wrapped in 0.9

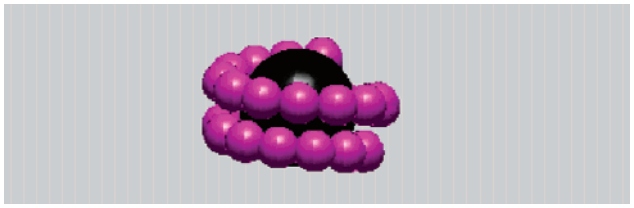

FIGURE 3: Compact form where 146 bp of DNA wrapped in 1.75 turns around the histone protein.

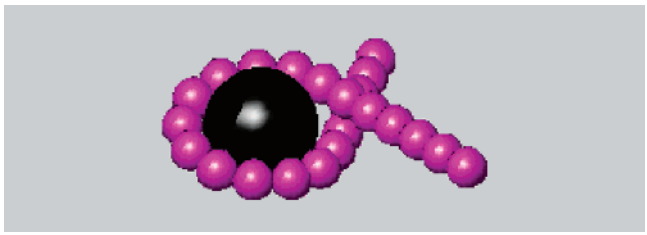

FIGURE 4: Stretched form where about 100 bp of DNA wrapped in 0.9 turn around the histone protein.

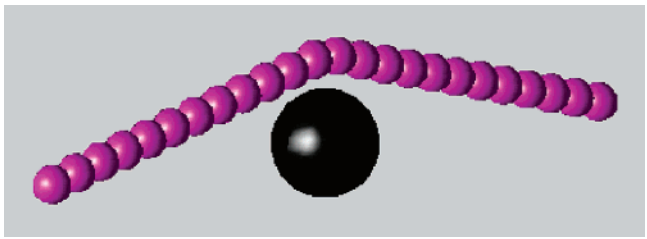

FIGURE 5: Loose form where no DNA wrapped around the histone protein.

turn around the histone protein as shown in Figure 4. This just means that raising temperature leads to stronger EDL effects in the surface of both DNA strands and histone protein. Upon further increasing the temperature, the histone−DNA complex enters the loose form, i.e., the third stage where no DNA strand wrapped around the histone protein as shown in Figure 5. In other words, the strong DNA strand−DNA strand repulsion potential prevents the histone−DNA complex.

The particular temperature range of 55−65 °C was worth thinking about twice, corresponding to no obvious unwrapping behavior between the singly wrapped stage and the twice-wrapped stage. This phenomenon provides us with a new insight into understanding nucleosome structure. It is true that the terminal regions of about 40 base pairs of DNA prefer to unwrap from the histone protein for higher temperature. We also know that the histone protein prefers to position at the DNA chain ending during the wrapping process (*17*). So a clear conclusion is that different regions of the DNA chain have different structures. The ending regions of the DNA chain are more sensitive to the physical parameters such as the temperature and salt concentrations, and thus the interaction between the histione protein with terminal regions of DNA will alter more dramatically with the change in surrounding solutions, compared with that of the center region of the DNA chain.

It should be noted that the end melting of DNA in this paper is unwrapping of terminal regions of DNA from the histone core particle with the increase in temperature, without any changing in structure and function of the DNA chain. Comparably, the melting of DNA is the denaturation of duplex DNA by heat or increased pH leading to strand separation.

CONCLUDING REMARKS

In this paper, the effects of temperature on the unwrapping behavior of DNA−histone complexes are analyzed. Our simulation results suggest two transitions in the shape of the nucleosome from a compact form where 146 base pairs of DNA wrapped in about 2 turns around the histone protein at low temperature into a more stretched form where about 100 base pairs of DNA wrapped up 1 turn, followed by the last loose form where the whole DNA chain escapes away from the histone core particle at higher temperature. The detailed thermodynamic investigation reveals the strongly discontinuous unwrapping behavior of the nucleosome by raising the temperature owing to the differences in structure and function between the ending region of the DNA chain and the center region of the DNA chain. Using Brownian dynamics, our results can qualitatively explain both static and dynamic features of nucleosomal particles, as well as ending effects or nucleosome sliding.

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