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Elucidating the DNA–Histone Interaction in Nucleosome from the DNA–Dendrimer Complex

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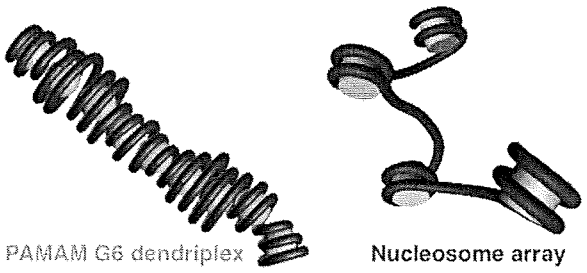
Supporting Information

ABSTRACT: The electrostatic complex of DNA with poly(amidoamine) G6 dendrimer (called "dendriplex") is used as a model system to resolve if pure electrostatic interaction can lead to the key structural features of nucleosome. Both dendrimer and histone octamer (HO) are found to attract DNA to wrap helically around them with comparable pitch lengths; however, the superhelical trajectory in the dendriplex is loose and fluctuating, whereas that in nucleosome is tight and rigid. The DNA-wrapped dendrimer particles are closely spaced along the dendriplex fiber, while the nucleosome core particles (NCPs) in the nucleosome array are separated by relatively long linker DNA. The clear contrast in structural features attests that DNA–HO interaction is beyond electrostatics, as additional specific interactions exist to fix DNA superhelical trajectory and to select the favored DNA sequence for constituting the NCP.



INTRODUCTION

The genome of eukaryotic organisms contains a great amount of genetic codes carried by DNA. The longest DNA in a chromosome can be as long as 2 m in the fully stretched length.[1] Since the cell nucleus in which the chromosomal DNA is accommodated is about 10 mm in dimension, the long DNA chains are believed to be compacted hierarchically with several levels to fit into the limited space of the nucleus. This problem, known as the "chromatin folding", is still under intensive study, as the detailed hierarchical structure associated with the DNA compaction has not been resolved completely.

Chromatin is composed of the basic building block called "nucleosome core particle" (NCP) which is interconnected by the linker DNA with different lengths.[2] NCP contains a histone octamer (HO) assembled by one H3–H4 histone tetramer and two H2A-H2B histone dimers and the nucleosomal DNA (~147 bp) that wraps around the HO with left-handed 1.75 turn superhelix of which the pitch length is 25.9 Å.[3,4] According to the intrinsic charges of DNA and HO, NCP can be regarded as an electrostatic complex of DNA polyanion and HO macrocation, with the net charges around −148e.[1,5] The fact that NCP is not perfectly charge neutral indicates that the nucleosomal DNA chain overcharges HO, and the electrostatic contribution to the free energy contains not only the interaction energy but also the entropic gain from the counterion release upon charge matching.[1,5−7]

In the chromatin fiber of 10 nm in diameter, the linker DNA separates the NCPs to form a linear array of nucleosomes, which is known as the "beads-on-a-string" (BOS) structure.[8] Such a "10 nm fiber" was postulated to undergo subsequent folding into the so-called "30 nm structure".[9] The length variation of linker DNA, which prescribes the position of the NCPs along the chromatin fiber, is related to the transcriptional activity of the genome.[10] It is believed that NCPs in chromatin are not positioned by chance but may be affected by some intrinsic (e.g., DNA sequence bendability[11,12] and the constraints from nucleosome packing[10,11]) and extrinsic factors (e.g., the interaction or binding with DNA-associated proteins such as the remodelers[13,14]). In fact, a special type of DNA, known as the Widom's "601" nucleosome positioning sequence (Widom 601 sequence), has been selected to demonstrate that NCPs can be positioned at specific sites *in vitro*.[15] Several studies have proposed that the position of the NCPs is a compromise among steric, attractive and repulsive interactions of the nucleosomes,[11] and the linker DNA is introduced by

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nature to allow the one-dimensional nucleosome array to fold into the compact fiber in a more efficient way.[16]

Poly(amidoamine) (PAMAM) dendrimer is a type of hyperbranched macromolecule comprising a core and layers of branches radiating from the core. The number of layers is called "generation number", denoted as Gn.[17] The grafting points at the interior of PAMAM dendrimer are the tertiary amine groups, whereas those on the outmost surface are the primary amine moiety. These amine groups can be positively charged by protonation under physiological or acidic condition; the resultant cationic dendrimer can then serve as a compaction agent for DNA[18] through forming the complex (called "dendriplex") by electrostatic interaction. Dendriplexes have received a significant amount of attention for the application in gene delivery.[19−25]

In addition to gene delivery, dendrimer is also of great interest from the aspect of biomimetics due to its geometric similarity to certain globule proteins. PAMAM G6 dendrimer with the diameter of ca. 67 Å is geometrically similar to HO. When one-third of the amine groups are protonated, PAMAM G6 dendrimer carries approximately the same charge as that of HO;[5,26] therefore, the complex of this specific dendrimer with DNA may serve as a simplified model system for facilitating the understanding of the fundamental problems associated with chromatin, including DNA−HO interaction, linker DNA formation, and chromatin folding. Both simulation[1,6,19,27] and experimental works[22,25,28−30] have demonstrated that, under sufficiently large charge density and generation number of the dendrimer, dendriplexes can form the chromatin-like BOS structure, in which DNA wraps around the dendrimer. Moreover, the complexation was found to follow the overcharging mechanism.[25,31]

Although the use of PAMAM dendrimer as a model HO has been proposed, no attempt has been made to compare the structural features of the dendriplex with those of the NCP or nucleosome array. In this study, we demonstrate that resolving the structural similarity and difference between these two systems can advance our understanding of nucleosome and chromatin in terms of the DNA wrapping mode governed by DNA−HO interactions and the controlling factor of linker DNA formation, which are important for deciphering the regulation mechanism of gene expression and the hierarchical compaction of chromatin. Using small-angle X-ray scattering (SAXS), we reveal the salient structural features of PAMAM G6 dendriplex, the NCP, and 12-mer nucleosome array. Through identifying the structural dissimilarity, we will show that DNA−histone interaction is beyond electrostatics, as additional specific interaction is operative to allow DNA to wrap tightly around HO to yield a rigid nucleosome structure; moreover, such an interaction may be sequence specific which renders HO the ability to select the DNA segment with the appropriate sequence to bind with HO.[32] The DNA sequences that are not selected by HO for binding are hence located outside the NCP and serve as the linker DNA.

EXPERIMENTAL SECTION

Dendriplexes Preparation. The PAMAM G6 dendrimer and Calf thymus DNA were purchased from Sigma-Aldrich. PAMAM G6 dendrimer, dissolved in deionized water (18.2 MΩ·cm) at room temperature (ca. 25 °C), was protonated by 0.1 N HCl(aq) to the prescribed degree of protonation (dp), which signifies the number fraction of protonated amine groups in the dendrimer. The dp values of the dendrimer were confirmed by the pH measurement. The protonated dendrimer solution was then titrated into the Calf thymus DNA aqueous solution (with the DNA concentration of 2 mg/mL) to reach the designated nominal N/P ratio, $(N/P)_n$, to form the dendriplex suspended in solution. The nominal N/P ratio stands for the feed molar ratio of the amine group of dendrimer to the phosphate group of DNA. In the present study, the dendriplexes with the $(N/P)_n$ of 6.0, 1.0, and 0.5 were prepared, which presribed the feed molar ratio of dendrimer to the base pairs of DNA of 1:43, 1:255 and 1:510, respectively. The range of feed molar ratio was sufficiently broad to cover that (1:147) associated with the NCP. Before the structure characterization experiment, the dendriplex was allowed to equilibrate for 72 h at room temperature. No further change in structure was observed with a longer equilibration time.

Nucleosome Core Particle (NCP) Reconstitution and Nucleosome Array Assembly. Both the NCP and 12-mer nucleosome array were prepared as described in previous studies.[33−35] The sequence of DNA in the 12-mer nucleosome array contained 12 177-bp repetition units that comprised a 147-bp high-affinity nucleosome sequence (the Widom 601 sequence) and a 30-bp sequence as a linker DNA.

Small Angle X-ray Scattering (SAXS). SAXS experiments were carried out at National Synchrotron Radiation Research Center (NSRRC) using PILATUS 1 M detector on BL23A S/WAXS beamline at λ = 0.1240 nm and the sample-to-detector distance of 3615 mm. The scattering profiles of NCP and 12-mer nucleosome array in buffer with the concentration of 2.5 and 9.63 mg/mL respectively, and those of the dendriplexes suspended in aqueous medium were collected under room temperature (ca. 25 °C). All scattering intensity profiles $I(q)$ were displayed as a function of scattering vector, q, with $q = 4\pi \sin(\theta/2)/\lambda$ (θ = scattering angle).

The program CRYSOL[36] was used to calculate the form factor of NCP from the crystallographic coordinates at 1.9 Å resolution.[37]

RESULTS

The structure of the dendriplex was studied as a function of dendrimer charge density expressed by the degree of protonation (dp), which signifies the number fraction of the protonated amine groups in the dendrimer. SAXS allows us to reveal the structural details of the dendriplex and the nucleosome systems through calculating the scattering profiles associated with the BOS structures using the coarse-grained models.[22,28−30] The X-ray scattering length density (SLD) of DNA, dendrimer and H_2O is 15.0×10^{10}, 11.3×10^{10}, and 9.3×10^{10} cm^{-2}, respectively. It can be seen that the X-ray SLD contrast of DNA relative to water is 2.85 times that of dendrimer; therefore, the SAXS intensity of the dendriplex suspended in water is dominated by DNA component.[28,29]

The SAXS profiles of the dendriplexes with the dendrimer dp values of 0.3, 0.5, and 0.7 are displayed in Figure 1a for the nominal N/P ratio of 6.0. All SAXS curves show two obvious peaks labeled as "q_d" and "q_P" in the q-ranges of 0.08−0.12 and 0.15−0.27 $Å^{-1}$, respectively. The features of the scattering patterns are analogous to those of the complexes with PAMAM G4 dendrimer with dp $\geq$0.6,[28] which was found to exhibit the BOS structure represented by the coarse-grained model in Figure 1b. In this model, the interconnection of the "nucleosome-like particles" formed by DNA wrapping around the dendrimer yields a chromatin-like fiber with wormlike global conformation. Different nucleosome-like particles are

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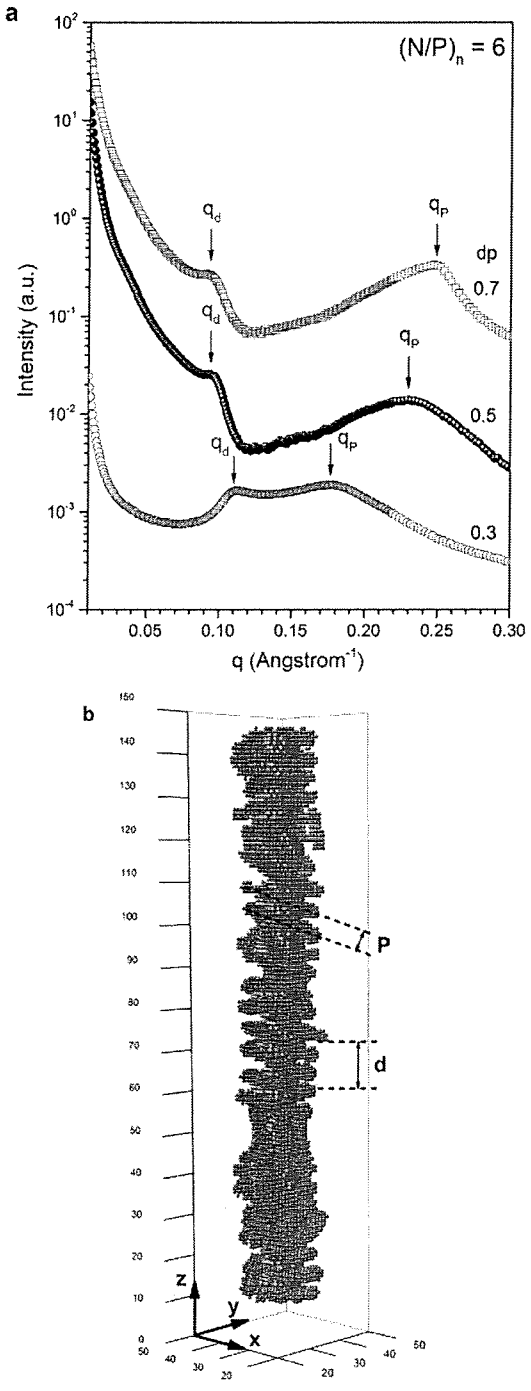

Figure 1. (a) Room-temperature SAXS profiles of the complexes of DNA with PAMAM G6 dendrimer with various dp values under the fixed nominal N/P ratio of 6.0. The SAXS profiles are vertically shifted for the clarity or presentation. (b) Wormlike chromatin-like fiber model used to calculate the SAXS profiles of the dendriplex. P is the pitch length of the DNA superhelix wrapping around dendrimer, and d is the distance between adjacent nucleosome-like particles.

linked by the "linker DNA" which is assumed to be rodlike.[22,28−30] On basis of this model, the q_d peak prescribes the average nearest-neighbor distance between the nucleosome-like particles, d, projected onto the fiber axis (i.e., z-axis) via $d = 2\pi/q_d$; the q_P peak is related to the pitch length (P) of the DNA superhelix wrapping around dendrimers via $P \approx 2\pi/q_P$. It is noted that the pitch peak appears clearly in the SAXS profile only when the coherent correlation between the helical segments spaced by the pitch length is strong.[28] This occurs when DNA wraps uninterruptedly around more than three dendrimer molecules along the chromatin-like fiber axis (see Figure S1 of the Supporting Information). That is, the helical trace of DNA has to continue over a sufficiently long distance to yield the q_p peak. If the wrapping is interrupted by relatively long linker DNA, the pitch peak is no longer observable. Thus, the presence of a clear pitch peak in the SAXS profile is an indicative of very short linker DNA between the nucleosome-like particles. It is noted that the scattering patterns similar to those displayed in Figure 1a were observed for the G6 dendriplexes with other nominal N/P ratios (see Figure S2) and also for the dendriplexes prepared from the same buffer solution (10 mM Tris pH 7.5, 10 mM KCl, 1 mM EDTA) as that used for preparing the nucleosome (see Figure S3).

The experimental SAXS profile of dp/0.5 dendriplex was compared with the SAXS profiles calculated from the wormlike chromatin-like fiber model with two distinct wrapping modes, i.e., the tight wrapping and the loose wrapping, as shown in Figure 2a. The tight wrapping model assumes that the DNA superhelix has a close contact with the surface of the dendrimer which is assumed to be spherical in shape, whereas the loose wrapping model allows the DNA superhelix to fluctuate in its radius (R_h) while wrapping around the dendrimer.[28,29] For the calculation of the SAXS profiles, we designated the value of one as the relative SLD contrast of dendrimer and the value of 2.85 as that of DNA.

Figure 2b and c display a snapshot of chromatin-like fiber with tight and loose wrapping mode, respectively. DNA was assumed to wrap around the dendrimers with a perfectly regular superhelical trajectory with the pitch length of 28 Å in the tight wrapping model and the interparticle distance between nucleosome-like particles was 69 Å. The calculated SAXS curve of this model is shown in Figure 2a along with the experimental scattering profile. It can be seen that the model predicts correctly the q_d peak at q = 0.09 Å^{-1} associated with the characteristic interparticle distance and a broad q_p peak at q = 0.22 Å^{-1} prescribed by the pitch length of the DNA superhelix. Both features agreed with those of the experimental SAXS profile of the dp/0.5 dendriplex. It is noted that the interparticle distance (d = 69 Å) of the nucleosome-like particles is close to the diameter (67 Å) of a single PAMAM G6 dendrimer molecule, showing that the linker DNA connecting two successive dendrimers was very short (<3 Å).

Apart from those features, the tight wrapping model yields a pronounced peak at q = 0.14 Å^{-1} (as marked by the dashed arrow) that was not observed experimentally. This peak originates from the first-order form factor of the DNA superhelix having a uniform and regular trajectory, and its position is prescribed by the average radius of the superhelix.[28] In order to smear this form factor peak of the superhelix, we introduced the loose wrapping model, which allows the trajectory of DNA wrapping around the dendrimer to fluctuate, such that its local radius, $R_h(z)$, varies randomly along the fiber axis (cf. Figure 2c). In the calculation, we set the maximum value of fluctuation of $R_h(z)$, $\Delta R_{h,max}$ = 20 Å, and the $R_h(z)$ value at each turn was assumed to be $R_h(z) = R_{h,0}(z) \pm \Delta R_h(z)$ with $R_{h,0}(z)$ being the radius associated with the tight-wrapping

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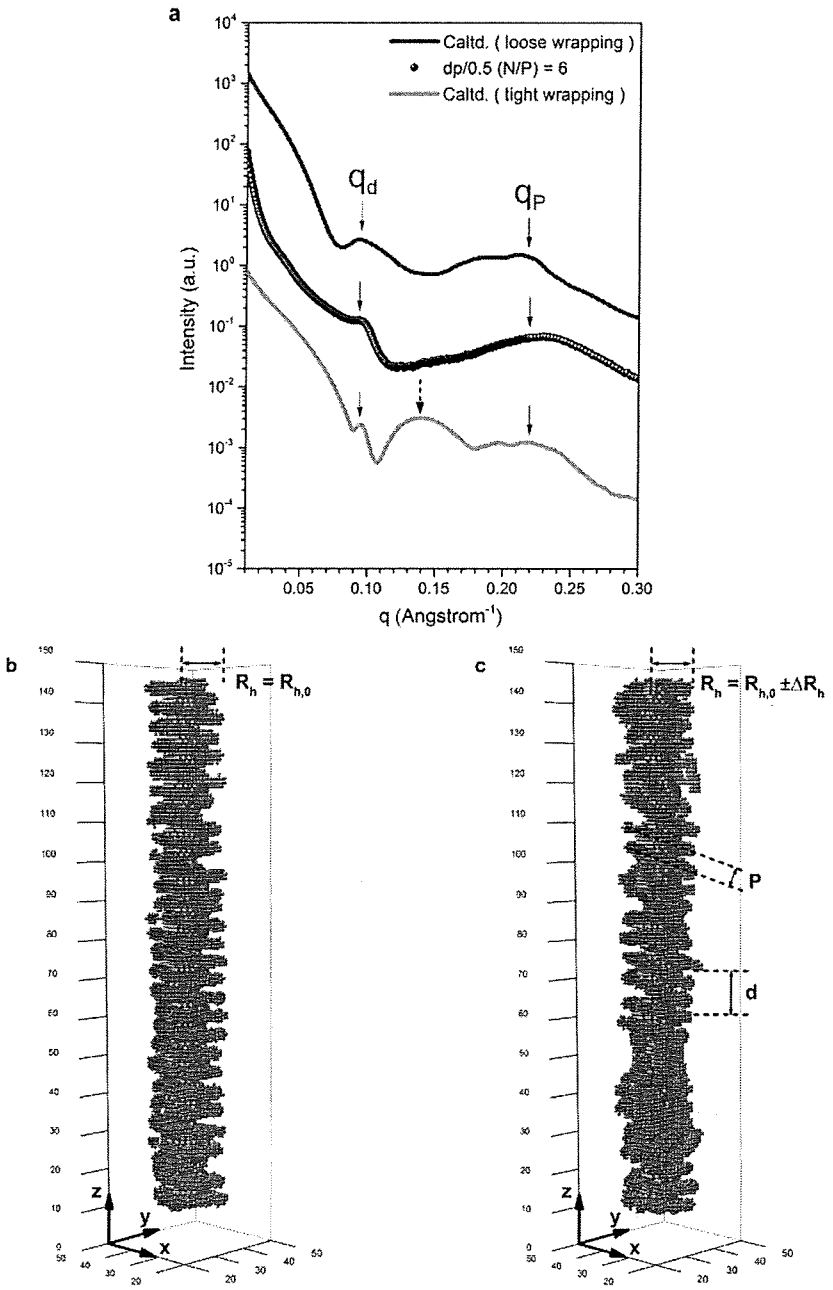

Figure 2. (a) Comparison between the experimentally observed SAXS profile and the calculated SAXS profiles using tight and loose wrapping model for the dp/0.5 dendriplex. The SAXS profiles are vertically shifted for the clarity or presentation. (b) Chromatin-like fiber model with the DNA wrapping around dendrimers tightly. (c) Chromatin-like fiber model with the DNA wrapping around dendrimers loosely.

superhelix and the value of ΔR_h being randomly assigned ($0 \leq \Delta R_h(z) \leq \Delta R_{h,max}$). The calculated SAXS curve based on the loose wrapping model displayed in Figure 2a now closely resembles the experimental profile, as the first-order form factor peak of the DNA superhelix was smeared and became hardly observable. The close agreement, which was also found in the other dendriplexes with different dp values (see Figure S4a and b in the Supporting Information), attests that DNA wraps around dendrimer loosely, with obvious fluctuations in superhelix radius.

Now we proceed to resolve the structural features of the nucleosome for comparison with those of the G6 dendriplex. Let us first consider the wrapping mode of DNA around a single HO in NCP. Figure 3a shows the experimental SAXS profile of NCP dispersed in the buffer solution (10 mM Tris pH 7.5, 10 mM KCl, 1 mM EDTA) at the concentration of 2.5 mg/mL. Our measured SAXS profile agreed with that reported in the literature, showing a broad shoulder at ca. 0.1 Å^{-1} and three peaks at 0.17, 0.23, and 0.3 Å^{-1}. The red curve superposing on the experimental data is the SAXS profile

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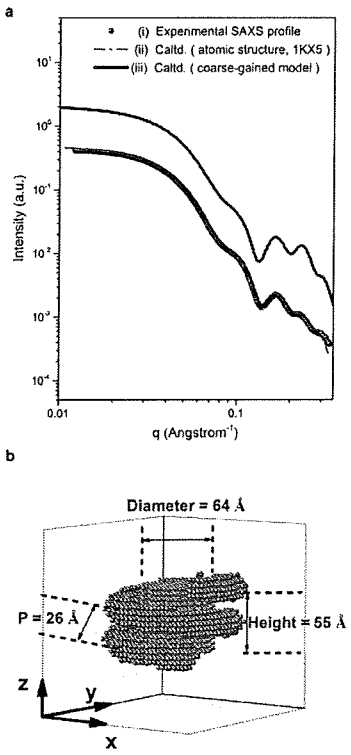

Figure 3. (a) Observed SAXS profile (i), the fitted result using the atomic structure (ii), and the calculated profile from the coarse-grained NCP (iii). The SAXS profiles are vertically shifted for the clarity or presentation. (b) Model of the coarse-grained NCP used for calculating the SAXS profile displayed by curve (iii) in part a.

calculated by the software CRYSOL[36] using the crystal structure of NCP (1KX5).[37] The profile calculated from this atomistic model agrees extremely well with the experimental result.

We further calculated the SAXS profile using a coarse-grained model for NCP (as shown in Figure 3b), which approximates a HO as a disk particle with the radius of 32 Å and the thickness of 55 Å, and DNA as a uniform helical cylinder wrapping tightly around the disk with the pitch length of 26 Å. Since the fluctuation of DNA superhelix was not considered in the calculation, the internal structure of NCP was assumed to be rigid in the coarse-grained model. For the intensity profile in the low-q region ($q < 0.1$ Å^{-1}), which is prescribed by the structural features at the large length scale, the experimental data matched the calculated scattering profile fairly well. At the higher q-region ($q > 0.1$ Å^{-1}), the calculated form factor of NCP also resembled the observed profile in terms of the agreement in the positions of the three peaks which correspond to the form factor maxima of the DNA superhelix. However, the calculated intensity was found to be higher than the observed value in the q-range between 0.135 and 0.35 Å^{-1}. This deviation may be caused by the assumption of uniform electron density of DNA in the constructed model, where the entire DNA chain was considered as a uniform helical cylinder with homogeneous electron density. The DNA chain in reality is a double helix and hence its electron density may vary along the wrapping trajectory.[38] Moreover, considering that there are only 14 contact points of DNA on HO in a NCP,[37] the wrapping trajectory of DNA may not follow that of the perfect helix prescribed by the mathematical formula adopted in the constructed model.[22,28,29] The imperfection of the trajectory may also cause the deviation of the calculated scattering profile from the observed one. In spite of the discrepancy in intensity in the higher q-region, the constructed coarse-grained model still offers a fairly good approximation for elucidating the structural features of NCP.

The fact that the observed SAXS profile of NCP was consistent with that calculated from its crystallographic structure attests to the fact that the internal structure of NCP in the solution was rigid, where the wrapping state of DNA in the NCP not only exhibited a significantly longer lifetime than the unwrapping state[1] but also showed very limited fluctuations in its superhelical trajectory.

Now we consider the scattering behavior of the chromatin fiber composed of 12 NCPs connected by linker DNA (called the "12-mer nucleosome array"). Figure 4a compares the

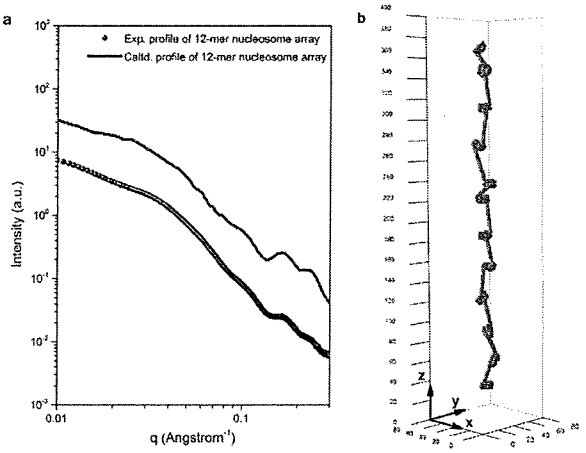

Figure 4. (a) Comparison between the observed and the calculated SAXS profile of 12-mer nucleosome array based on the coarse-grained model depicted in part b. The SAXS profiles are vertically shifted for the clarity or presentation. (b) Model of the 12-mer nucleosome array used to calculate the SAXS profile displayed in part a. The distance between NCPs is 110 ± 50 Å and each NCP has identical geometric characteristics to that of the NCP displayed in Figure 3b.

experimentally observed SAXS profile of the 12-mer nucleosome array with the profile calculated by the model of wormlike chromatin fiber composed of 12 coarse-grained NCPs (as presented in Figure 4b) connected by the rodlike linker DNAs with the length of 110 ± 50 Å. It can be seen that the calculated SAXS profile matched the experimental profile well at the low-q region ($q < 0.08$ Å^{-1}), where the global conformation of the chromatin fiber dominates the scattering intensity; however, the calculated intensity was higher at the larger q (0.08 Å$^{-1} < q < 0.3$ Å^{-1}). In fact, the observed scattering profile at $q > 0.06$ Å^{-1} closely resembled the form factor scattering of a NCP presented in Figure 3a; therefore, the deviation of the calculated intensity profile from the observed one was caused by the same reasons as those proposed for the single NCP system. It is noted that the pitch peak, which was observed for PAMAM G6 dendriplex, was not identified for the nucleosome array. This fact along with the close similarity of the high-q intensity profile to NCP form factor attest that the NCPs in the chromatin fiber were separated by long linker DNAs, such that the scattered waves from the segments of the

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DNA superhelices at different NCPs were not strongly correlated. This is in clear contrast to the PAMAM G6 dendriplex in which the nucleosome-like particles were closely spaced along the fiber direction.

DISCUSSION

The electrostatic complexes of PAMAM dendrimer with DNA have been studied for the interests of gene delivery,[39−42] electrostatic compaction of DNA[20,21,23,43] and the construction of nanostructured templates.[44−46] Previous works have revealed that the structure of the dendriplex depends on the dendrimer generation number, the charge density of dendrimer (dp), the salt concentration of the medium, and the nominal N/P ratio. Once the generation number of dendrimer is sufficiently high (G$n \geq$ G4), the dendriplex is able to form the nucleosome-like BOS structure under certain condition.[19,22,23,25,28,29] On the basis of the fact that the PAMAM G6 dendrimer is geometrically similar to HO and its charge can be adjusted to the level equivalent to that of HO, it is plausible to use the G6 dendriplex as a model system to examine if pure electrostatic interaction contributed by the electrostatic attraction energy and the entropic gain from counterion release can lead to the key structural features of the NCP or even chromatin fiber.

Therefore, here we undertake a comparison between the structure of G6 dendriplex and that of the nucleosome array. We revealed that, under the dp values ($0.7 \geq$ dp ≥ 0.3) and nominal N/P ratios ($6.0 \geq (N/P)_n \geq 0.5$) studied, DNA was able to wrap around the dendrimer to form the BOS structure, of which the pitch length of the DNA superhelix was about 25 to 30 Å. BOS structure is a well-established characteristic of the chromatin fiber comprising NCP and linker DNA. Nevertheless, there are clear differences between the BOS structures of these two systems. In the dendriplexes, where the interaction is predominantly electrostatic in nature, DNA wraps around dendrimers with a loose superhelix and a fluctuating trajectory. However, the observed SAXS profile of NCP was fitted very well by its atomic crystal structure and also matched the SAXS curve calculated from the coarse-grained model of the particle in which a superhelical cylinder wraps around a disk with a regular and nonfluctuating trajectory. The fluctuations of R_h in the dendriplex may arise from the thermal fluctuations of the monomer units constituting the dendrimer molecule; however, the fact that the fluctuations of R_h also exist obviously in the dendriplex with G9 dendrimer[29,30] (which also exhibited smearing of the first-order form factor peak of the DNA superhelix) indicates that electrostatic interaction alone is not sufficiently strong to fix the trajectory of the DNA superhelix even when it is allowed to wrap around a relatively rigid body (i.e., G9 dendrimer). Thus, the fluctuations of DNA superhelix arising from the action of thermal fluctuations is a natural consequence as long as the interaction is purely electrostatic in nature under moderate dendrimer charge density.

By contrast, the DNA in the NCP was found to exhibit a stable trajectory with a sustained pitch length (~25.9 Å) and a well-defined number and position of contact points with HO.[3,37] This difference in wrapping mode suggests that there are additional forces besides the electrostatic interaction to regulate the wrapping process of DNA in NCP, such that HO can compact DNA in an explicit and efficient way. In fact, fluctuations will disturb the selection of the target DNA sequence for transcription; nature has to suppress the fluctuation effect by introducing additional specific interaction between DNA and HO. The crystal structure of NCP reveals that there are 14 binding sites between HO and DNA, which are hydrogen bonding in nature.[3,37] Since these binding sites are located at well-defined positions on HO,[3,4] the collective interaction from hydrogen bonding may be responsible for fixing the DNA superhelical trajectory in NCP and solidifies its overall structure. Previous studies have shown that, upon applying an external force on the two ends of the DNA superhelix in NCP and the chromatin fiber, the NCP and the nucleosomes in the fiber exhibited a multistage unwrapping behavior, where the force required to unwrap the outmost turn is smaller than that associated with the inner turn.[47−49] This discontinuous unwrapping behavior may also offer the evidence showing that the interaction between HO and DNA is not governed by the electrostatic force exclusively.

The present SAXS study also disclosed the distance between the adjacent nucleosome-like particles in G6 dendriplex to be around 57 to 69 Å, which was close to the diameter of a single PAMAM G6 dendrimer. This finding attests that DNA not only wraps around the dendrimer loosely but also gathers the dendrimer molecules into a compact arrangement along the fiber axis. That is, there is no pronounced linker DNA between adjacent dendrimer molecules in the chromatin-like fiber formed by the dendriplex. The close packing of the nucleosome-like particles in the dendriplex was further verified in real space by the AFM micrograph in Figure 5, which shows

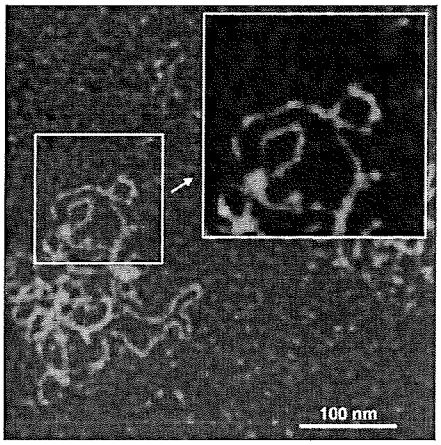

Figure 5. Representative AFM topographic image of PAMAM G6 dendriplex cast on mica surface under air atmosphere. The morphology of dendriplex was characterized by long fibers or chains collapsed into quite compact globules. The height of the fiber was about 2 nm. One selected region was enlarged at the upper right corner with its contrast adjusted to clearly demonstrate the dense packing of the beads along the fiber.

the topographic image of the dendriplex cast on the mica surface. It can be seen that the morphology of the dehydtrated dendriplex was characterized by the long fibers or chains which collapsed into quite compact globule. The average height of the fiber was about 2 nm, which was reasonable considering that the height of the dehydrated DNA chain and PAMAM G6 dendrimer on mica surface observed by AFM was about 0.7[50,51] and 0.4−0.7 nm,[2,4] respectively. A close examination of the local texture revealed that the fibers were composed of interconnected beads that were closely spaced without obvious linker DNA between the successive beads. The observed real-space

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morphology was consistent with the SAXS results showing the absence of long linker DNA.

In the case of 12-mer nucleosome array, the average distance between the neighboring NCPs was found to be about 110 Å. This large spacing between NCPs was also manifested by the fact that there was no discernible pitch peak in the corresponding SAXS profile; consequently, the NCPs in the nucleosome array were well separated and connected by relatively long linker DNAs.

For the complex of highly charged spherical macroions with a single chain bearing opposite charge under the condition of low salt concentration (such that the Debye screening length is large comparing to the radius of spherical macroion) and large Manning parameter (>1), it has been proposed that the spherical macroions will overcharge the oppositely charged chain due to the fact that the overcharging process may liberate more counterions that were originally condensed on the macroions. In this case, the number of spherical macroions wrapped by the polyelectrolyte chain is larger than that prescribed by the isoelectric point, such that the distance between adjacent macroions is reduced into a negligibly small value.[6] According to the length of the DNA wrapping around a dendrimer macrocation, the DNA-wrapped dendrimer particles were indeed negatively charged, as the number of the negatively charged phophaste groups of DNA wrapping around the dendrimer was always larger than that of the positively charged ammonium groups of dendrimer over the dp range studied.[52] If the two negatively charged particles were interconnected by a long linker DNA bearing a higher charge density, one would expect a stronger electrostatic repulsion between the particles and the linker DNA than the direct repulsion between two closely spaced particles without the interconnecting DNA.

Since both the dendriplex and the 12-mer nucleosome array can be regarded as the complexes of a linear DNA polyelectrolyte and macrocations, the complexation processes should follow the overcharging process if the electrostatic free energy dominates. Nevertheless, only the dendriplex system exhibited close packing of the macrocations along the chromatin-like fiber, while the nucleosome array showed deviation from the overcharging behavior, as the individual NCPs were isolated by relatively long linker DNA. This finding implies that a driving force stronger than the counterion release may exist to introduce long linker DNA between the NCPs.

It has been believed that the nucleosomal DNA and the linker DNA in the chromatin are composed of the "favored" and "unfavored" sequences, respectively.[10] The most favored sequence is constituted of the periodic pattern of AA/AT/TT dinucleotides and GC dinucleotides, which are inserted in the middle region between two adjacent AA/AT/TT dinucleotides with a period of approximately 10 bp. It was postulated that such a sequence could be bent at a lower energy cost and is hence energetically favored for wrapping around HO.[10,11,13] The segments in DNA without the favored sequence are excluded from nucleosome and form the linker DNA. Although the sequences of the DNAs adopted in the two system were different, where the DNA in the 12-mer nucleosome array was specifically designed to contain the Widom 601 sequence, while the DNA in the dendriplexes was the native calf thymus DNA without further modification, the overall sequence-independent properties such as the persistence length and the charge density of the DNAs should be identical. Moreover, the native calf thymus DNA, which was extracted from the chromosome of calf thymus, should also contain the favored and unfavored sequences constituting nucleosomal DNA and linker DNA, respectively.[10,53] The absence of linker DNA in the dendriplex implies that the selection of favored and unfavored sequences was not effective; that is, the bendability-controlled phenomenon was not operative in the dendriplex wherein the component interaction was mainly electrostatic in origin. We propose that the sequence selection that leads to long linker DNA in chromatin is operated by HO instead of being governed by the intrinsic bendability of DNA sequence. That is, HO has the ability to select the appropriate sequence to bind tightly with DNA to form NCP, whereas dendrimer does not, such that the wrapping of DNA around the dendrimer is driven predominantly by electrostatic interaction, which leads to essentially no linker DNA between the nucleosome-like particles.

It is known that a certain type of proteins, known as the "DNA-binding proteins", can exhiit specific affiity to DNA via a mixed mechanism which allows the proteins to read the base sequence or the shape (conformation) of DNA.[54−57] Two types of mechanism are believed to be operative, namely, the formation of hydrogen bonds with specific bases in the major groove of DNA and the sequence-dependent deformation of DNA helix. The present study suggests that HO belongs to such a class of protein. In fact, it has been proposed that HO could detect the local variation in DNA shape and electrostatic potential to achieve base-specific binding with DNA.[58] The present work attests that studies along the line of exploring the mechanism of sequence recognition by HO is one of the most crucial tsaks for understaning the nucleosome and chromatin formation.

■ CONCLUSION

This study has thus taken a step in the direction of identifying the role of the interaction between HO and DNA in forming nucleosomes by using a simplified model system: the PAMAM G6 dendrimer−DNA complex. Our finding indicates that both dendrimer and HO have the ability to attract DNA chain to wrap around them with a comparable pitch length; however, the wrapping trajectory in dendriplex system is loose and fluctuating, while DNA wraps around HO through a sustained trajectory with very limited fluctuations. Furthermore, the dendrimers in the dendriplexes were gathered closely by DNA to achieve the free energy minimum by means of overcharging process, while the NCPs in the 12-mer nucleosome array were well separated by relatively long linker DNA. Our results suggest the existence of additional specific interactions beyond electrostatics between HO and DNA in nature to fix the DNA superhelix around HO and to select the favored DNA sequence to form the NCP.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.6b00311.

SAXS profiles of the dendriplexes with other nominal N/P ratios and dendrimer dp values, comparisons between the SAXS profiles calculated from the BOS model and the observed scattering profiles of the dendriplexes with various dendrimer dp values, calculated SAXS profiles associated with the beads-on-structure formed by the continuous wrapping of DNA around a number N of

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dendrimer macrocations, and SAXS profiles of the dp/0.3 dendriplex prepared in pure water and in the same buffer solution (10 mM Tris pH 7.5, 10 mM KCl, 1 mM EDTA) as that used for preparing the nucleosome (PDF)

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Notes

The authors declare no competing financial interests.

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