Beads-on-String Structure of the Electrostatic Complex of DNA with a High-Generation PAMAM Dendrimer

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Abstract. The electrostatic complexes of polyanionic DNA with cationic dendrimer have been considered as a potential non-viral vector for gene delivery and a model system for understanding DNA-histone interaction. Although it is believed that the gene transfection efficiency may be influenced by the structure of the complex, the supramolecular structure of DNA-dendrimer complexes and its dependence on various system parameters such as dendrimer generation number, charge density, charge ratio and ionic strength are not well resolved. In this study, we investigate the structure of the complex of DNA with polyamidoamine (PAMAM) dendrimer of generation nine (G9) by means of synchrotron small angle X-ray scattering (SAXS). It is found that DNA is always able to wrap around the dendrimer to yield the beads-on-string structure irrespective of the charge density of the dendrimer. The effect of charge density on the persistence length of the chromatin-like fiber thus formed and the pitch length of the DNA superheix wrapping around the dendrimer are elucidated from the calculation of the SAXS profiles based on beads-on-string structure models.

1. Introduction

The electrostatic complexes of polyanionic DNA with various cationic agents, including lipids, macrocations, polyelectrolytes and amphiphilic block copolymers, have received much attention in recent years due to the effort in developing non-viral vectors for gene therapy. [1] The complexation is driven mainly by the electrostatic attraction between DNA and the cationic species coupled with the entropic gain from counterion release and it usually results in significant aggregation of DNA chains, leading to the formation of submicrometer-sized particles. [2,3] Two levels of the structure can hence be defined for the complexes: (1) the “colloidal level” characterized by the topological feature (e.g. the shape and size) and the surface charge of the particles at the length scale of several hundred nm or above; (2) the “supramolecular level” characterized by the organization of DNA chains and the cationic agent within the particles at the characteristic length scale of several nm. It is believed that the gene transfection efficiency is influenced by the structure of the complex; [4,5] consequently, resolving the structures at both levels and the strategy for tuning them by various parameters such as charge ratio, ionic strength, pH and temperature have been regarded as an important fundamental task for the realization of effective non-viral gene vector.

The present study concerns the supramolecular structure of the complexes of DNA with cationic dendrimer (called “dendriplexes”) in pure water. Dendrimer is a class of hyperbranched macromolecule composing of layers of monomer units irradiating from a central core. [6] Each
complete grafting cycle is called a “generation” (denoted by Gn with n being the generation number). The dendrimers possessing amine groups at the surface and/or the interior can be protonated to controlled level under acidic aqueous environment. The macocations thus formed have been considered as the carriers for macromolecular drug and gene delivery. [7]

Based on the above discussion, it is suggestive that the complexes of DNA with high-generation dendrimers (e.g. G7 ~ G9) may exhibit the beads-on-string structure, while those with low-generation dendrimers (e.g. G2 and G3) may show the ordered columnar mesophase. The more complicated systems will hence be the complexes with dendrimers of intermediate generations (e.g. G4 ~ G6). In this case, neither the DNA bending energy nor the electrostatic interaction may completely dominate the structure formation; therefore, the dendriplexes may exhibit interesting structure transformation with respect to the interplay between these two factors prescribed by the charge density of the dendrimer, the charge ratio, salt concentration, etc. Moreover, some structures intermediate between the columnar mesophase and the beads-on-string structure may be formed. Using synchrotron small angle X-ray scattering (SAXS), we have shown recently that, depending on the charge density of the dendrimer prescribed by its degree of protonation (dp), the complexes of DNA with PAMAM G4 dendrimer exhibited three distinct nanostructures characterized by different degrees of DNA bending. [17] At dp < 0.3 the dendriplex displayed a square columnar phase, while the beads-on-structure with DNA wrapping around each dendrimer tightly by ca. 1.4 turns was formed at dp ≥ 0.6 . An intermediate structure called “hexagonally-packed DNA superhelices” was identified at 0.3 ≤ dp ≤ 0.5, where the DNA chains organized in a hexagonal lattice twisted moderately to enhance the charge matching with the dendrimer. The observed structural transition with respect to the increase of dendrimer dp was in accord with the increasing weighting of electrostatic attraction over DNA bending energy.

In this paper, we investigate the structure of the complex of DNA with PAMAM G9 dendrimer to examine if DNA can always wrap around this high-generation dendrimer with various charge densities due to lower DNA bending energy cost. Some previous attempts have been made to reveal the wrapping of DNA around dendrimer. For example, Ottaviani et al. used electron paramagnetic resonance (EPR) spectroscopy to study the interactions between dendrimers and DNA and nitroxide-labeled PAMAM dendrimers. [18,19] DNA chain was found to wrap around G7 dendrimer. Similarly, Chen et al. investigated the binding of dendrimers to ctDNA by fluorescence titration of Ethidium bromide (EB) with a premixed solution of DNA and various amount of dendrimers. [20] The DNA complexation with PAMAM G7 dendrimer was found to be analogous to the DNA-histone interaction as DNA could wrap around the dendrimer. These spectroscopic studies could reveal the local interaction of DNA with dendrimer; however, the knowledge of the “beads-on-string” structure at a larger length scale remains largely unknown. Previous AFM studies have observed the globule particles formed by DNA-dendrimer complex, indicating that the DNA chain adsorbed on the surface of dendrimer could be bent due to strong electrostatic interaction. [21] Although detailed internal structure of these particles was not observed by AFM, Ritort et. al. used force-extension curves (FECs) to show similar unfolding and refolding force as well as similar compaction ratios to those of chromatin fibers. [22]

In this study, synchrotron SAXS is employed to gain insight into the structure information ranging from the internal structure of the DNA-wrapped dendrimer particles to the global organization of these “nucleosome-like” particles. We will demonstrate that DNA chain is indeed able to wrap around G9 dendrimer tightly even for the dendrimers with extremely low charge density. It will also be shown that the global organization of the resultant nucleosome-like particles or the conformation of the “chromatin-like” fibers is influenced by the charge density of the dendrimer.

2. Experimentals
2.1 Materials. Linear Calf Thymus DNA was purchased from ICN and used without further purification. Its molecular weight was ca. 9.2 kbps. Ethylenediamine (EDA) core polyamidoamine G9
dendrimer was obtained from DNT as methanol solutions. After thorough drying, the solids were weighed and then redissolved in distilled water to produce a stock solution of 0.1% (w/w). The solutions were stored at 4 °C till use.

2.2 Complex Preparation. To complex with the polyanionic DNA, the amine groups in PAMAM dendrimer were first protonated by adding prescribed amount of 0.1 N HCl solution. The primary amine groups at the outer surface of the dendrimer tended to be protonated first because their basicity (pKₐ ≈ 9.0) is larger than that of the interior tertiary amines (pKₐ ≈ 5.8). Therefore, the PAMAM G9 dendrimers with different degrees of protonation (dp) were prepared by controlling the pH of the solution. The solution of the protonated dendrimer solution was then mixed with the aqueous solution containing prescribed amount of DNA to obtain the complex. The concentration of DNA aqueous solution was 2 mg/ml. The complexation was usually manifested by visually observable precipitation.

2.3 Small Angle X-ray Scattering (SAXS) Measurements. The supramolecular structures of the complexes in pure water were probed SAXS at room temperature. The aqueous suspensions of the complexes were directly introduced into the sample cell comprising of two ultralene windows. The SAXS experiments were performed at the Endstation BL23A1 of the National Synchrotron Radiation Research Center (NSRRC), Taiwan. The energy of X-ray source and sample-to-detector distance were 14 keV and 2259 mm, respectively. The scattering signals were collected by MarCCD detector of 512x512 pixel resolution. For the in-house experiment, the wavelength of X-ray source and sample-to-detector distance were 0.154 nm (CuKα) and 65 cm (for the low-q configuration) or 23 cm (for the high-q configuration), respectively. The scattering intensity profile was output as the plot of the scattering intensity (I) vs the scattering vector, q = (4π/λ)sin(θ/2) (θ = scattering angle), after corrections for sample transmission, empty cell transmission, empty cell scattering and the detector sensitivity.

3. Results and discussion

The charge density of the PAMAM G9 dendrimer is prescribed by its dp value which stands for the number fraction of protonated amine groups in the dendrimer. The nominal N/P ratio of the dendriplex, prescribed by the feed molar ratio of the amine groups (irrespective of whether they are protonated) to the phosphate groups of DNA, is fixed at 6/1.

Figure 1 displays the SAXS profiles of the dendriplexes with three dp values for the G9 dendrimer. The scattering patterns are very different from those associated with the ordered columnar phases exhibited by the dendriplexes with lower-generation dendrimers, [22] showing that DNA in the complexes does not organize to form columnar mesophases. For dp=0.02 and 0.1, the SAXS pattern is characterized by a shoulder (marked by “qₘ”) near 0.05 Å⁻¹, a small hump (marked by “i=1”) at ca. 0.08 Å⁻¹ and a large broad shoulder/peak near 0.14–0.15 Å⁻¹. When the dp is increased to 0.5, the SAXS profile displays a sharper primary peak and a small shoulder (marked by “i=1”) appearing like a form factor peak. An additional peak (marked by “qₚ”) is identified at 0.24 Å⁻¹.

Because the ordered columnar mesophase is not formed in DNA/G9 complexes, we resort to another possible structure, namely, the “beads-on-string” structure. A G9 dendrimer molecule (R = 59 Å) is
much larger than a lower-generation dendrimer molecule, such as G2 (R = 12 Å) thereby rendering a much higher charge density in protonated G9 dendrimer (cf. 2048 positive charges on G9 vs. 16 positive charges on G2 for dp = 0.5). The strong electrostatic attraction between DNA and G9 dendrimer may induce the DNA chain to wrap tightly around the dendrimer molecule for effective charge matching. The energy cost in bending the DNA chain to wrap around a G9 dendrimer is also lower since the large molecular size reduces the surface curvature of the dendrimer.

It is known that the subunit of chromatin, called “nucleosome”, consists of a 147-bp DNA wrapping around a 7 nm cationic octamer of histone protein. This kind of beads-on-string structure of chromatin or isolated nucleosome particles has been characterized by SAXS and SANS. We hence first consider the salient features of the scattering behavior of chromatin from the literature and then examine if the scattering behavior of DNA-G9 complex is analogous to that of chromatin.

Baldwin et al. reported a series of SANS profiles of chromatin dispersed in D$_2$O/H$_2$O mixture (the concentration of chromatin in the solution was 50 wt%) for contrast variation experiment. [24] According to the authors, the SANS pattern collected at 10% D$_2$O approximates to the SAXS pattern. In this case, the scattering profile shows a low-q peak and two additional high-q peaks. The low-q peak with the equivalent Bragg spacing (d) of ca. 10.5 nm was attributed to the interparticle distance between the nucleosome particles constituting the chromatin fiber. The two high-q peaks were considered to stem from DNA component, where the peak corresponding to d = 5.5 nm was ascribed to the pitch of DNA wrapping around the histone protein. The observed SAXS patterns of dp 0.02 and 0.1 dendriplexes studied here are indeed quite similar to that SANS profile of chromatin, which strongly suggests that the DNA chain wraps around G9 dendrimer to yield the beads-on-string structure (as schematically illustrated in Figure 2) even at the dp as low as 0.02 (corresponding to 82 positive charges per dendrimer macrocation). Since the DNA used here has 9200 base pairs with the fully extended length of ca. $3 \times 10^{-5}$ m, each DNA strand is able to wrap around a large number of G9 dendrimer, giving rise to the “chromatin-like fiber” composing of the “nucleosome-like particles”.

Although the scattering result implies the formation of beads-on-string structure irrespective of dp, the fact that the SAXS profile depends on dp indicates that the internal structure of the chromatin-like fiber varies with dp. Following the work of Baldwin et al., [24] we consider the primary peak to stem from the spatial correlation of the nucleosome-like particles. The interparticle distance calculated from the peak position ($q_m$) via $d = 2 /q_m$ is 12.6 nm. Considering that the diameters of DNA and dendrimer are 2.0 nm and 11.4 nm, respectively, the interparticle distance of the closely packed nucleosome-like particles should be $d = 11.4 + 2 \times 2 = 15.4$ nm. The observed $d$ is however smaller than this value. We postulate that the primary scattering peaks in SAXS profiles are instead associated with the correlation of the dendrimer molecules along the fiber contour (i.e. z-axis; see Figure 2). The dendrimer molecules are closely spaced along the fiber axis as the interparticle distance is only slightly larger than the molecular diameter.
The chromatin-like fiber formed by the complex should possess certain persistence length. The fact that the primary peak is broad and weak in intensity at dp < 0.5 signals that the axial correlation is limited and hence the fiber has a relatively small persistence length (or more flexible). The primary peak becomes sharper and more intense when dp is increased to 0.5, implying that the complex fiber becomes stiff with large persistence length. In this case, the charge density of the dendrimer is very high (with 2048 charges on the surface), and a significant amount of positive charges remain unmatched even if DNA wraps around the dendrimer tightly. The complex is hence overcharged and the strong electrostatic repulsion between the overcharged nucleosome-like particles stiffens the fiber significantly. Moreover, the long-range correlation of the pitches due to persistent wrapping of DNA along the fiber axis leads to a relatively sharp and clear pitch peak located at ca. 0.24 Å⁻¹. The pitch length calculated from the peak position according to \( P = \frac{2\pi}{q_p} \) is 2.6 nm. This value is slightly larger than the diameter of DNA, showing that the DNA segments are closely spaced along the fiber contour to effectively match the positive charges on dendrimer at the cost of bending energy.

The formation of beads-on-string structure by DNA-G9 dendrimer complexes is formally verified by comparing the observed SAXS profiles with the calculated form factor of a chromatin-like fiber. We construct a chromatin-like rod formed by a DNA chain wrapping around a number of dendrimer macrocations placing along a fiber axis (i.e., z axis) with the axial interparticle distance of \( d \) (Figure 2). Each dendrimer is approximated by a sphere with internal monomer density fluctuations, \([25]\) and the DNA superhelix is approximated by a uniform helical cylinder with a prescribed pitch length \( P \) and pitch angle, as shown in Figure 3. The radius of the superhelix (\( R_h \)) given by \( R_h = P/(2\pi \tan \theta) \) is defined as the radial distance between the centerline and central trace of the helix (\( R_h = 0 \) for completely straightened DNA, see Figure 3). Therefore, the helical trace of the cylinder can be calculated from the following equations.\([26]\]

\[
x(z) = R_h \sin \left( \frac{2\pi z}{P} + \phi \right); \quad y(z) = R_h \cos \left( \frac{2\pi z}{P} + \phi \right)
\]

where \( \phi \) is phase angle that prescribes the direction of the groove of the superhelix. The regular pitch of a helix can give rise to a scattering peak locating at \( q_p = \frac{2\pi}{P}l_p = 2\pi P \cos \theta \), where \( l_p \) is the projection of \( P \) onto the normal of the helical segment (see Figure 3). It can be shown that \( q_p \approx 2\pi l_p \) as long as \( 2\pi R_h \) is significantly larger than \( P \). It is noted that the wrapping of DNA around the dendrimer is assumed to be tight; therefore, the value of \( R_h \) may vary with \( z \).

After constructing the chromatin-like fiber with prescribed values of \( P \) and \( d \), we divide the system into numerous volume elements (each with the size of 8 x 8 x 8 Å³) and the partial structure factors associated with DNA-DNA correlation \( [S_{DD}(q)] \), dendrimer-dendrimer correlation \( [S_{dd}(q)] \) and

Figure 4. Comparison between the experimentally observed SAXS profile and the calculated SAXS pattern for the dp/0.5 dendriplex. The SAXS profile is calculated assuming a chromatin-like rod composing of 10 nucleosome-like particles placing in sequence with \( P=2.6 \) nm and \( d = 14 \) nm. The partial structure factors associated with DNA-DNA and dendrimer-dendrimer correlation, i.e. \( S_{DD}(q) \) and \( S_{dd}(q) \), are also displayed. The figure on the right shows the actual picture of the chromatin-like segment generated for calculating the SAXS curves.
DNA-dendrimer correlation \([S_{dd}(q)]\) of the randomly oriented chromatin-like fiber are calculated by the Debye equation\(^\text{27}\)

\[
S_{nm}(q) = \frac{1}{N^2} \sum_{i=1}^{N} \sum_{j=1}^{N} \sin\left(\frac{q|\mathbf{r}_i - \mathbf{r}_j|}{q|\mathbf{r}_i - \mathbf{r}_j|}\right)
\]  

(1)

where \(n\) and \(m\) stands for either DNA (\(D\)) or dendrimer (\(d\)) and \(|\mathbf{r}_i - \mathbf{r}_j|\) is the distance between \(i\) and \(j\) volume element. \(S_{dd}(q)\) is further corrected by adding an additional component arising from internal monomer density fluctuations.\(^\text{25}\) The SAXS intensity is finally calculated from the three partial structure factors by

\[
I(q) = \Delta \rho_D^2 S_{DD}(q) + 2\Delta \rho_D \Delta \rho_d S_{DD}(q) + \Delta \rho_d^2 S_{dd}(q)
\]  

(2)

where \(\Delta \rho_D (= 5.7 \times 10^{-10} \text{ cm}^2)\) and \(\Delta \rho_d (= 2 \times 10^{-10} \text{ cm}^2)\) is the scattering length density contrast of DNA and PAMAM dendrimer relative to water, respectively. It is noted that at present we have not been able to obtain the scattering profiles that quantitatively match the experimental results, as there are a large number of parameters needed to be considered, such as the distribution of SLD within the dendrimer molecule, the possible size distribution of dendrimer arising from non-uniform protonation, the persistence length of the chromatin-like fiber, the wrapping mode of DNA, etc. Here we merely seek the structures that give rise to the scattering profiles resembling the experimental results, and from which we can identify the salient features of the beads-on-string structures formed at different degrees of protonation.

Figure 4 compares the experimental SAXS profile of the dendriplex with \(dp = 0.5\) with that calculated for a chromatin-like rod composed of 10 nucleosome-like particles placing in sequence with \(P = 2.6\) nm and \(d = 14\) nm. The partial structure factors associated with DNA-DNA and dendrimer-dendrimer correlations, i.e., \(S_{DD}(q)\) and \(S_{dd}(q)\), are also displayed in the figure. From the calculated SAXS profile, it can be seen that the scattering pattern at \(q < 0.12\ \text{Å}^{-1}\) is dominated by \(S_{dd}(q)\), where the primary peak is associated with the interparticle distance between the dendrimers (or the nucleosome-like particles) along the \(z\) axis and the small hump near 0.08 Å\(^{-1}\) is the first form factor maximum of the dendrimer. However, \(S_{dd}(q)\) dominates the scattering pattern at \(q > 0.12\ \text{Å}^{-1}\). In this case, a clear pitch peak \((q_P)\) becomes visible at 0.24 Å\(^{-1}\). The intensity and breadth of this peak is dependent on the number of nucleosome-like particles in the fiber assumed for the calculation. The peak drops in intensity and broadens with decreasing number of nucleosome-like particles. Therefore, a chromatin-like fiber with larger persistence length should display a more clear pitch peak along with a sharper primary peak in the SAXS profile.

The close resemblance of calculated SAXS profile to the experimentally observed scattering pattern verifies the formation of beads-on-string structure by the dp/0.5 dendriplex. The calculation also confirms that the primary peak is associated with the axial correlation of the dendrimer (or nucleosome-like particle) in the

![Calcd. SAXS Profile (\(P=2.8\) nm; \(\sigma=0.3\) nm)](exp.sxs.profile(dp=0.2)

**Figure 5.** Comparison between the experimentally observed SAXS profile and the calculated SAXS pattern for the dp/0.2 dendriplex. The SAXS profile is calculated assuming a chromatin-like rod composing of four nucleosome-like particles with a Gaussian distribution of the pitch length (mean value = 2.8 nm and the variance =0.3 nm).
chromatin-like fiber and the peak marked by “i = 1” corresponds to the first form factor maximum of the dendrimer. The peak observed at 0.24 Å⁻¹ corresponds to the pitch peak found in the calculated profile; therefore, the clear pitch peak in the observed SAXS profile attests a rather large persistence length of the chromatin-like fiber and the pitch length of the DNA wrapping is 2.6 nm. Our present calculation is however not rigorous enough to provide an accurate estimate of the persistence length.

For dendriplexes with lower dp, no clear pitch peak is identified and the corresponding SAXS profiles in the high-q region display a broad shoulder. Moreover, the primary scattering peak is also broad and relatively weak. These features attest that the persistence length of the chromatin-like fiber is short. We found that the assumption of a monodisperse pitch length in the nucleosome-like particles cannot produce the SAXS profile showing a broad high-q shoulder. On the other hand, the assumption of polydisperse pitch length yields the SAXS pattern closely resembles the experimentally observed profiles. Figure 5 displays the experimental SAXS profile of the complex with dp = 0.2 and the SAXS profile calculated under the assumption of Gaussian distribution of the pitch length with the mean value of 2.8 nm and the variance of 0.3 nm. In this case, the intensity at q > 0.05 Å⁻¹ is obtained by summing the form factor profiles of the nucleosome-like particles with different pitch lengths according to the weighting prescribed by the Gaussian distribution function. The low-q intensity (q < 0.05 Å⁻¹) is calculated for the chromatin-like rod composing of four nucleosome-like particles placed in sequence with the interparticle distance of 11.8 nm. It can be seen that the agreement is fairly good, thereby showing that the chromatin-like fiber formed by the dendriplex with lower dp is more flexible (comparing to that associated with dp/0.5 dendriplex) and there exists a relatively clear distribution of pitch length of the DNA superhelix wrapping around the dendrimer.

4. Conclusion

We have revealed that DNA can wrap around PAMAM G9 dendrimer to yield the chromatin-like structure irrespective of the charge density of the dendrimer. The wrapping mode of the nucleosome-like particle and the global conformation of the chromatin-like fiber however depend on dendrimer charge density. At low dp (< 0.5), DNA can still wrap around the dendrimer tightly with a distribution of pitch length; the chromatin-like fiber thus formed has a smaller persistence length. At dp = 0.5, the DNA chain wraps around the dendrimer regularly and tightly with the pitch length of 2.6 nm. The resultant chromatin-like fiber is highly stiff due to strong electrostatic repulsion between the nucleosome-like particles.

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6. References

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