

The Effects of Mechanical Properties of DNA on the Thermodynamic Stability of the DNA-Dendronized Polymer Nanocluster

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The effects of the mechanical properties of DNA, such as bending, twisting, and bending-twisting interactions on the thermodynamic stability of DNA-dendronized polymer nanoclusters with different conformations in terms of free energy are investigated. The effect of temperature on the free energy is also studied and the values of enthalpy and entropy of the solution of the nanocluster are predicted. The obtained thermodynamic quantities help us to have a better understanding about the mechanical properties of DNA and the stability of the nanocluster in gene therapy.

Keywords: DNA-dendronized polymer nanocluster, Bending free energy, Twisting free energy, Electrostatic free energy, Gene therapy, Elastic free energy

INTRODUCTION

Gene therapy offers the potential to cure a wide range of diseases [1,2]. Two of the most common carriers for gene delivery are viral [3] and synthetic vectors [1,2,4,5]. Viruses are efficient carriers, but the safety concerns and difficult large-scale production limit the application of viral vectors [6]. Consequently, there is intense activity in developing and evaluating synthetic nonviral vectors, including liposome, polylysins, cationic linear polymers and, recently, dendrimers [7,8].

Compactification of DNA into a large nanocluster (100-nm) with oppositely charged polycations is an important tool in gene therapy to deliver DNA into an infected cell [9]. Physical and chemical properties of dendrimers are an

outgrowth of their shape as well as the presence of charged group on their surface. The dendrimer's high positive charge density, without immunogenicity or carcinogenicity [10], together with high solubility in water and high efficiency for *in vitro* use, led to its use as an efficient medium for DNA transfer. It is also possible to change the charge and structure of the dendrimer to modify its properties for transfection [11]. For example, the dendronized polymers are new type of dendrimer, which are cylindrical in shape and capable of producing a nanocluster (inclusion aggregate) with DNA in the solution. The nanocluster is formed by wrapping the DNA around the dendronized polymer [12-14]. Electron microscopy photographs of dendronized polymers and nanoclusters have revealed their cylindrical shape [12].

The required free energy for DNA of a specific arrangement to aggregate with a known dendronized polymer can be computed by breaking it up into mechanical and electrostatic interaction terms. The mechanical properties of DNA originate from its elastic behavior while undergoing

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external forces. At low external forces, DNA is a semi-flexible polymer [15] and, at high external forces, DNA can suddenly be driven to an almost fully stretched state [16]. Electrostatic and elastic models of DNA have been widely investigated [15-27].

Different models have been proposed to describe the elastic behavior of linear polymers. The random walk is the simplest model, where the polymer path is modeled by a sequence of uncorrelated steps in space [20]. This model is appropriate for polymers of simple chemical structures or for polymers with very long chains. More complex polymers exhibit a certain bending stiffness that preserves the direction of the polymer over a persistence length. This leads to the classical model of an inextensible semi-flexible polymer or worm-like chain model, which is well adapted to DNA.

In our previous works [13-14,28-33], the electrostatic potential generated around the nanocluster has been numerically calculated by solving the nonlinear Poisson-Boltzmann equation. The electrostatic potential and then the electrostatic free energies, entropic free energies and distribution of counter ions have been determined for a series of DNA-dendronized polymer nanoclusters at different temperatures and ionic strengths.

In the present work, we investigate the effect of the mechanical properties of DNA on the thermodynamic stability of the nanocluster. The effects of bending, twisting and bending-twisting interactions are studied for different conformations of nanoclusters at different temperatures at a constant ionic strength. The free energy, enthalpy and entropy of the solution are predicted, giving us a better understanding of the thermodynamic stability of the nanocluster.

Dendronized polymers are a new class of synthesized dendrimers and their nanoclusteration with DNA has not yet been experimentally investigated. Therefore, as far as literature investigations are concerned, there is no experimental data available with which to compare our results. On the contrary, our theoretical results, based on physical principles, are useful to the experimentalist, giving guidance in experimental design.

Theory and Computational Procedure

The following are two major factors affecting the stability of the nanocluster:

Electrostatic contribution. Nasehzadeh *et al* [13,14,28-33] have previously provided a clear picture and an appropriate definition of the DNA-dendronized polymer nanocluster. A model for the nanocluster has been designed and the electrostatic potentials and electrostatic free energies for charging the nanocluster have been calculated using the nonlinear Poisson-Boltzmann equation. The electrostatic contribution to the thermodynamic stability of the nanocluster has then been discussed. In this work we use the procedure of Gaossel *et al.* [13].

Elastic contribution. The elastic energy associated with the rigidity of a flexible rod disfavors the helical conformation, thus this form of energy, related to the conformation of a rod, can be parameterized by arc length (s), which is defined as the length along a curve. We describe the rod by relating its local coordinate frame L to its reference frame L_0 , rigidly embedded in the curve in its relaxed configuration at each point of the arc length. The helical conformation is parameterized [24] as:

$$\bar{\mathbf{r}}(s) = \begin{pmatrix} R \cos\left(\frac{2\pi s}{\eta\sigma}\right) \\ R \sin\left(\frac{2\pi s}{\eta\sigma}\right) \\ \frac{s}{\eta} \end{pmatrix} \quad (1)$$

where R is the radius of wrapping η is the length of DNA per unit length of dendronized polymer, and σ is the height per turn.

The bending and twisting free energies of a rod with the length ℓ are related to the helical conformation. The configuration of an inextensible polymer, such as a helical conformation, is specified by three orthonormal unit vectors $\{\mathbf{u}(s), \mathbf{n}(s), \mathbf{t}(s)\}$ along the chain, where \mathbf{t} is the axial direction vector of the DNA double helix and \mathbf{u} is a unit vector perpendicular to \mathbf{t} and pointing from one backbone chain to the other, $\mathbf{n} = \mathbf{t} \times \mathbf{u}$. It proves to be convenient to use Euler angles by setting $\mathbf{e}_1 = \mathbf{u}$, $\mathbf{e}_2 = \mathbf{n}$, and $\mathbf{e}_3 = \mathbf{t}$, with $\partial \mathbf{e}_i / \partial s = \boldsymbol{\Omega} \times \mathbf{e}_i$, where $\boldsymbol{\Omega} = (\Omega_1, \Omega_2, \Omega_3)$ is angular velocity of the frame $\{\mathbf{e}_i\}$. These three unit vectors can be driven for a helix by using Eq. 1:

$$\mathbf{t} = \frac{d\mathbf{r}(s)}{ds} = -\left(\frac{2\pi R}{\eta\sigma}\right) \sin\left(\frac{2\pi s}{\eta\sigma}\right) \mathbf{i} + \left(\frac{2\pi R}{\eta\sigma}\right) \cos\left(\frac{2\pi s}{\eta\sigma}\right) \mathbf{j} + \frac{1}{\eta} \mathbf{k} \quad (2)$$

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$$\mathbf{u} = \frac{d\mathbf{t}/ds}{|d\mathbf{t}/ds|} = -\text{Cos}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{i} - \text{Sin}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{j} \quad (3)$$

$$\mathbf{n} = \mathbf{t} \times \mathbf{u} = \frac{1}{\eta} \text{Sin}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{i} - \frac{1}{\eta} \text{Cos}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{j} + \left(\frac{2\pi R}{\eta\sigma}\right)\mathbf{k} \quad (4)$$

For a helix the expression ($\partial \mathbf{e}_i / \partial s = \boldsymbol{\Omega} \times \mathbf{e}_i$) can be written as:

$$\frac{d\mathbf{t}(s)}{ds} = (\Omega_1 \mathbf{i} + \Omega_2 \mathbf{j} + \Omega_3 \mathbf{k}) \times \left(-\left(\frac{2\pi R}{\eta\sigma}\right) \text{Sin}\left(\frac{2\pi s}{\eta\sigma}\right) \mathbf{i} + \left(\frac{2\pi R}{\eta\sigma}\right) \text{Cos}\left(\frac{2\pi s}{\eta\sigma}\right) \mathbf{j} + \frac{1}{\eta} \mathbf{k} \right) \quad (5)$$

$$\frac{d\mathbf{u}}{ds} = (\Omega_1 \mathbf{i} + \Omega_2 \mathbf{j} + \Omega_3 \mathbf{k}) \times \left(-\text{Cos}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{i} - \text{Sin}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{j} \right) \quad (6)$$

$$\frac{d\mathbf{n}}{ds} = (\Omega_1 \mathbf{i} + \Omega_2 \mathbf{j} + \Omega_3 \mathbf{k}) \times \left(\frac{1}{\eta} \text{Sin}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{i} - \frac{1}{\eta} \text{Cos}\left(\frac{2\pi s}{\eta\sigma}\right)\mathbf{j} + \left(\frac{2\pi R}{\eta\sigma}\right)\mathbf{k} \right) \quad (7)$$

Then, using Eqs. 5-7, it is possible to derive expressions for the angular velocities.

$$\Omega_1 = 0, \Omega_2 = \frac{2\pi}{\eta\sigma} \left(\frac{2\pi R}{\eta\sigma} \right) \text{and } \Omega_3 = \frac{1}{\eta} \left(\frac{2\pi}{\eta\sigma} \right) \quad (8)$$

The most general elastic energy up to quadratic order in the deformations should be of the form

$$\frac{\Delta G_{elas}}{kT} = \Delta G_{bend} + \Delta G_{twis} + \Delta G_{bend-twis} = \int_0^\ell ds \left[\frac{A'}{2} \Omega_1^2 + \frac{A}{2} \Omega_2^2 + \frac{C}{2} \Omega_3^2 + B \Omega_1 \Omega_3 \right] \quad (9)$$

The first two terms are related to bending deformations. A' and A are bending persistence lengths along the directions \mathbf{e}_2 and \mathbf{e}_1 , respectively. The third term is twisting energy, the last term is caused by bend-twist coupling, and B is the coupling constant. The $\Omega = 0$ (see Eq. 8) indicates that the bending deformation is only along the direction \mathbf{e}_1 and, therefore, it is clear that the bending and twisting deformation is independent and bending twisting coupling must be eliminated to calculate elastic free energy. The bending and twisting free energies, which are obtained from Ω_2 and Ω_3 , are then given by:

$$\Delta G_{bend} = \frac{A}{2} \ell R^2 \left(\frac{2\pi}{\eta\sigma} \right)^4 \quad (10)$$

$$\Delta G_{twis} = \frac{C}{2} \left(\frac{2\pi}{\eta^2 \sigma} \right)^2 \ell \quad (11)$$

The total free energy of nanocluster is then given by:

$$\Delta G_{total} = \Delta G_{elec} + \Delta G_{elas} \quad (12)$$

The enthalpy and entropy of nanocluster can be also calculated by using the Gibbs-Helmholtz equation.

$$\left(\frac{\partial \Delta(G/T)}{\partial (1/T)} \right)_p = \Delta H \quad (13)$$

We previously developed the finite-difference computational procedure to calculate ΔG_{elec} [13], which is also used in this work. The free energies of bending and twisting were calculated by using Eqs. 10 and 11, using previously published values for the dielectric constant of solvent (water) at different temperatures [34].

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of temperature on the electrostatic free energy (bending and twisting free energies excluded) and total free energy (bending and twisting free energies included) of nanoclusters with different conformations (different temperatures are stated in the insets of the figures in degrees Kelvin). From these figures we can see that, as the temperature varies, the stability of the nanoclusters changes, with the most stable configuration corresponding to the least free energy at the lowest temperature. In Fig. 1, the most thermodynamically stable conformation corresponds to the value of pitch = 32 Å; however, while the bending and twisting free energies are included, this value will be shifted to 35 Å (Fig. 2). These figures also indicate that the variation of temperature has no effect on the minimum free energies.

The effect of temperature on the total free energy (application of the Gibbs-Helmholtz equation) is shown in Fig. 3. The slope of this plot gives us the total enthalpy of the nanocluster, $\Delta H_{total} = -586 \text{ kJ mol}^{-1}$, which indicates that the charging process of nanocluster is exothermic.

Different values of enthalpy for different conformations of the nanocluster are presented in Fig. 4, which shows that the enthalpy decreases as the size of the pitch increases (*i.e.* as the size of the pitch increases, more heat is released). This reveals that by increasing the size of the pitch, the adsorption of mobile ions onto the nanocluster is increased. The entropies of nanoclusters with different conformations are negative and decrease as the size of the pitch increases (see Fig. 5). This figure indicates that the system becomes more

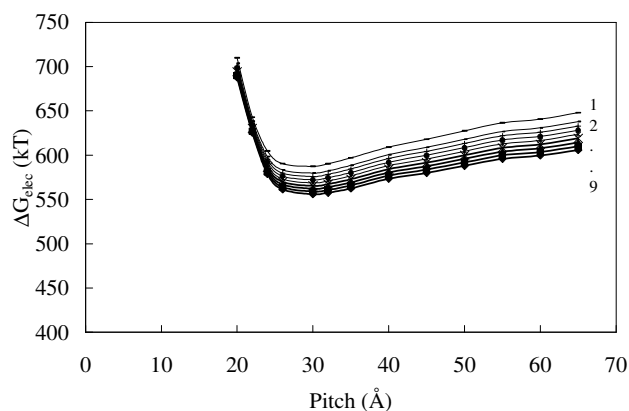


Fig. 1. The effect of temperature (K) on the electrostatic free energy of nanoclusters with different conformations: (1) 323.15, (2) 313.15, (3) 308.15, (4) 303.15, (5) 298.15, (6) 293.15, (7) 288.15, (8) 283.15, (9) 278.15.

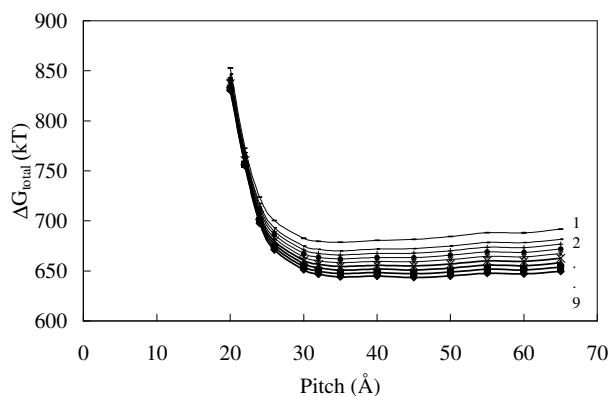


Fig. 2. The effect of temperature on the total free energy of nanoclusters with different conformations: (1) 323.15, (2) 313.15, (3) 308.15, (4) 303.15, (5) 298.15, (6) 293.15, (7) 288.15, (8) 283.15, (9) 278.15.

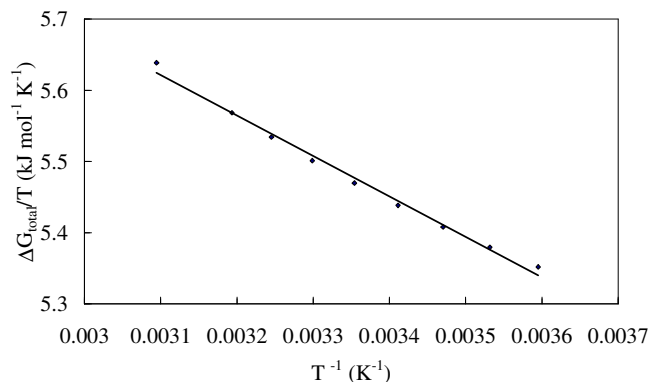


Fig. 3. The effect of temperature on the total free energy of nanocluster with conformation of pitch 35 Å: $y = -567.95x + 7.3822$ ($R^2 = 0.9924$).

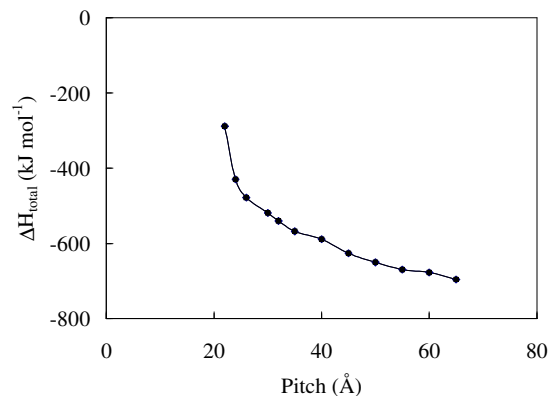


Fig. 4. Variation of enthalpy with the size of pitch.

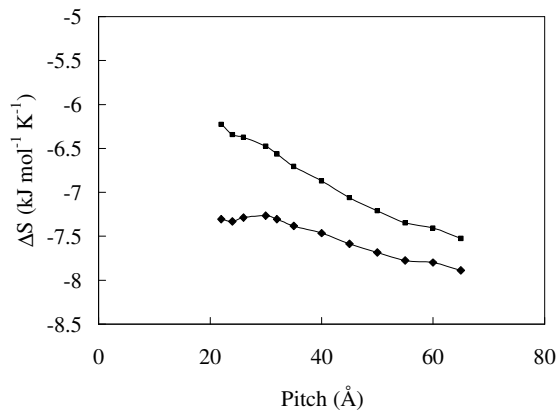


Fig. 5. Variation of entropy with the size of pitch: (◆) total, (■) electrostatic.

ordered as the size of the pitch increases. This reveals that, as the size of the pitch increases, more space on the surface of the nanocluster will be available to counter mobile ions attracted to the nanocluster.

CONCLUSION

By solving the Poisson-Boltzmann equation using the finite-difference numerical method, electrostatic free energies are determined for DNA-dendronized polymer nanoclusters with different DNA pitches. The sum of elastic free energy and electrostatic free energy gives us the total free energy of the nanocluster system, which could be used to obtain the optimum conformation at constant ionic strength (0.1 M). The total enthalpy and entropy of nanocluster were then calculated by using Gibbs-Helmholtz equation. It is concluded that the elastic free energy will destabilize the nanocluster, especially at a lower pitch. In addition, temperature variations have an appreciable effect on the electrostatic free energy.

SYMBOLS

k	Boltzmann constant
T	Absolute temperature
ΔG_{elec}	Electrostatic free energy
ΔG_{elas}	Elastic free energy
ΔG_{total}	Total free energy
ΔG_{twis}	Twisting free energy
ΔG_{bend}	Bending free energy
\vec{r}	Position vector
A, A'	Elastic constants of bending
B	Elastic constant of bending-twisting interaction
C	Elastic constant of twisting
s	Arc length
η	Length of DNA per unit length of dendronized polymer
σ	Height per turn
R	Radius of wrapping
ℓ	Length of DNA
$\mathbf{u}(s), \mathbf{n}(s), \mathbf{t}(s)$	Three orthonormal unit vectors
$\Omega = (\Omega_1, \Omega_2, \Omega_3)$	Angular velocity of the frame

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