Effects of protein binding on topological states of DNA minicircle *

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Abstract. Two models of nonspecific protein binding on dsDNA are proposed: DNA-bending protein and DNA-stiffening protein. In both cases, protein binding enhances the bending rigidity of dsDNA at its binding site, but the DNA-bending protein will introduce a bending angle. According to the models mentioned above, by taking Metropolis-Monte Carlo algorithm, the effects of nonspecific protein binding on the topological states of DNA minicircle are discussed systematically. The writhe number distribution changing with chemical potential and the direct conformation with protein binding is demonstrated. At the same time, the autocorrelation of segment and the average binding number of protein changing with chemical potential are addressed. Some insights into the protein-DNA binding mechanism are obtained.

Keywords: Metropolis-Monte Carlo algorithm, mechanism of protein binding, DNA-binding protein, DNA-stiffening protein

1 Introduction

DNA-binding proteins play a central role in all aspects of genetic activities within an organism, such as transcription, packaging, rearrangement and replication and repair. So it is critical to examine the nature of complexes that are formed between proteins and DNA. As they provide the basis of our understanding of how these processes take place. Until now on the basis of a structural analysis of 240 protein-DNA complexes contained in the Protein Data Bank, the DNA-binding proteins involved can be classified into eight different structural or functional groups, which can be further classified into fifty-four structural families[7]. Some nonspecific proteins have been examined extensively, such as HU protein, H-NS protein. Recent experiments[1–3, 9, 11] show that, for short DNA molecule (1000bp), HU-DNA complexes will be formed into thick, rigid filaments while incubated into 900nM HU (1 dimer per 1.8bp), but when the DNA molecule with the same length is incubated in 18nM HU, the protein will induce bending-angle, and the angle distribution is rather broad. While super-coiled circular DNA incubated in HU with saturating amounts (1 dimer per 9bp), relaxed conformations are found, which reveals that the forming of protein-DNA complexes depend on the concentration of protein and the protein binding will affect the conformation of closed circular DNA. Here two models of protein-binding on DNA are proposed, in the following sections, the average occupation number protein, direct conformation with protein binding and so on, will be discussed based on the two models proposed above, some insights into the mechanism that protein binds on DNA are shown.

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2 Model and theory

In our simulation, we consider the DNA molecule consisting of \( n \) straight segments where the conformational energy is carried by the bending of the vertices connecting the adjacent segments. The energy of this model is a summation of the vertex energy, of equilibrium length \( b \), each segment contains three base pairs. The conformation is described by the orientations of the segments \( \hat{t}_i \), where \( i = 1, \ldots, n \) and the conformational energy is carried by the bending of the vertices connecting the adjacent segments. The energy of this model is a summation of the vertex energy,

\[
E = \sum_{i=1}^{n-1} E_i(\hat{t}_i, \hat{t}_{i+1})
\]  

(1)

2.1 Discrete model of DNA-Bending proteins

For semi-flexible polymer model of DNA, while the protein is binding on DNA, the bending energy at the binding site can be expressed as:

\[
\beta E_i = \frac{\alpha}{2} |\hat{t}_{i+1} - \hat{t}_i|^2 (1 - n_i) + \left[ \frac{\alpha'}{2} (\hat{t}_{i+1} - \hat{t}_i - \gamma)^2 - \mu \right] n_i
\]  

(2)

where in \( \beta = (k_B T)^{-1} \), the terms \( \alpha \), \( \alpha' \), and \( \mu \) in Eq. (2). In both cases, \( n_i = 1 \), the protein binds on DNA, otherwise, no protein binds on DNA, the bending rigidity of protein-bound and bent DNA and the binding free energy of DNA-binding proteins. When a protein binds on DNA and locally force a bend by an angle \( \psi \), we often use \( \cos \psi \equiv \gamma \) in our simulation.

2.2 Discrete model of DNA-stiffening protein

Another case is that when a protein binds, it only enhance appreciably its bending stiffness does not bend dsDNA. This case can be described as:

\[
\beta E_i = \frac{\alpha}{2} |\hat{t}_{i+1} - \hat{t}_i|^2 (1 - n_i) + \left[ \frac{\alpha'}{2} |\hat{t}_{i+1} - \hat{t}_i - \gamma|^2 - \mu \right] n_i
\]  

(3)

The terms \( \alpha \), \( \alpha' \) and \( \mu \) in Eq. (3) are same to the ones in Eq. (2). In both cases, \( n_i \) denotes two binding states 0 or 1, while \( n_i = 1 \), the protein binds on DNA, otherwise, no protein binds on DNA.

2.3 The writhe number definition

Writhe number is a parameter describing the three-dimensional folding of helical DNA, which can be obtained from the double-contour integral,

\[
W_R = \frac{1}{4\pi} \int_0^1 \int_0^1 \frac{\hat{t}(s_1) \times \hat{t}(s_2) \cdot (\hat{r}(s_1) - \hat{r}(s_2))}{|\hat{r}(s_1) - \hat{r}(s_2)|^3} ds_1 ds_2
\]  

(4)

where the expression for the trajectory is \( \hat{r}(s) \), \( \hat{t}(s) = \frac{d\hat{r}}{ds} \) is unit tangent vector of the trajectory. The writhe number is commonly approximated by discrete Gauss integral\[^4\,\,10\]

\[
W_R = \frac{1}{4\pi} \sum_{i=1}^N \sum_{j=1, i \neq j}^N \hat{e}_{ij} \cdot \hat{e}_i \frac{\hat{r}_i - \hat{r}_j}{|\hat{r}_i - \hat{r}_j|^2}
\]  

(5)

where in \( \hat{r}_i - \hat{r}_j \) denotes the positions of origins of the \( i \)th and \( j \)th segments, respectively. The unit vector along the vector \( \hat{r}_i - \hat{r}_j \) is \( \hat{e}_{ij} = \frac{\hat{r}_i - \hat{r}_j}{|\hat{r}_i - \hat{r}_j|} \).

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2.4 Algorithm

On the simulation, we take the Metropolis-Monte Carlo procedure, which is used for the statistical sampling of chain conformations. In the process of the procedure, chain parts were displaced consecutively. At very step, whether the new trial conformation is rejected or accepted or not, all the conformations must be added to the conformations series. The initial conformation can be chosen arbitrarily. In order to generate the new trial conformations, a type of displacement can be performed. The subchain rotated by a randomly chosen angle, around the straight line connecting two randomly chosen vertexes in the chain. Whether the trial conformation is accepted or not can be determined by the metropolis rules: If the new trial conformation energy $E_{new}$ is lower than the old conformation energy $E_{old}$ then the trial conformation is accepted. Otherwise, the trial conformation was accepted by the probability $\exp\left[\frac{(E_{old} - E_{new})}{k_B T}\right]$.

3 Results and discussion

3.1 The average occupation number of protein binding on the DNA minicircle under the discrete model of DNA-bending proteins

Fig. 1 ∼ Fig. 4 address the average occupation number of protein binding on 40nm minicircle changes with the chemical potential of protein binding. With the chemical potential ranging from -3.91 to 6.91, the average occupation number increases critically approximating to 40. For the chemical potential 6.91, during the simulation procession, the average occupation number fluctuates in the range from 39.93 to 40, which means that except few sites, all forty sites in the 40nm-minicircle are bound by proteins. At other chemical potentials, the 40nm-minicircle is bound partially by proteins. Bending angle also has obvious effects on the average occupation number of proteins binding on the DNA minicircle, from the comparison between Fig. 5 and Fig. 2 the effects of bending angle will be found Both Fig. 5 and Fig. 2 have the same length of DNA minicircle and chemical potential, but the bending angle in Fig. 5 is $\pi/2$ and the one in Fig. 6 the effects of chemical potential are very obvious, with the chemical potential increasing, the average binding number increases sharply, which is same to theoretical results\[5\]. By the way, same work has been done on the 80nm-DNA minicircle, the results are similar to 40nm-DNA minicircle. The average occupation number changing with the chemical potential will have obvious effects on autocorrelation of segments direct conformation of DNA-minicircle and the writhe number distribution shown in the following sections. Fig. 1.

![Fig. 1.](image1)

![Fig. 2.](image2)

number of 40nm-minicircle changes with chemical potential of protein binding, wherein the discrete model of DNA-bending proteins is taken, in Fig. 1 ∼ Fig. 4 and Fig. 6, the bending angle is $\pi/3$, the bending angle in Fig. 5 is $\pi/2$.

3.2 The direct conformation with protein binding changes with chemical potential under the discrete model of DNA-bending proteins

Generally, short stiff double-strand DNA (dsDNA) can be smoothly cyclized into circular. The conventional theory of cyclization of short DNA uses semi-flexible polymer model, the bending energy of a DNA
molecular of length $L$ is taken to be that of thin-beam elastic theory:

$$\frac{E}{K_BT} = \frac{A}{2} \int_0^L ds \left( \frac{\partial \hat{t}}{\partial s} \right)^2$$

where $\hat{t}(s)$ is tangent vector at arc length position $s$ setting the energy in $k_BT$ unit makes the dimension of the elastic constant $A$ a length, it is called persistence length, about 50nm. The energy required to bend a DNA of length into a smooth circular is $E_{/k_BT} = 2\pi^2 A/L$, when $L$ is comparable to or smaller than the persistence length 50nm (150bp), the energy become large compared to $K_AT$, while $L = 40nm, 80nm$, the energy required is about $25k_BT, 12k_BT$. As a result, it is hard to be folded further into other conformations for short DNA-minicircle. While protein binding exits, the DNA-minicircle can be folded further. Effects of protein binding on DNA-minicircle conformation are demonstrated in Fig. 7 ∼ Fig. 10, Protein binding introduces $\pi/3$ bending angle, minicircle can be folded further into different conformations, and 40nm-length minicircle and 80nm-length minicircle are taken as example. On the condition that the length of DNA minicircle is fixed, with the chemical potential increasing, the effects of protein folding become more obvious, which reveals the powerful functions of protein during life procession. The direct conformations of DNA minicircle with protein binding are addressed by discrete model of DNA-bending proteins. In Fig. 7, Fig. 8, the direct conformation of 40nm-length with bending angle $\pi/3$ change with chemical potential; In Fig. 9 and Fig. 10, the direct conformation of 80nm-length with bending angle $\pi/3$ change with chemical potential, in both cases, chemical potential are 6.91, 3 respectively.

### 3.3 Autocorrelation of segments changes with chemical potential of protein binding under the discrete model of DNA-bending proteins

The correlation function in continuous-tangent model can be addressed as $\langle \hat{t}(s) \cdot \hat{t}(s') \rangle = e^{-|s-s'|/A}$. Normally, for a DNA-minicircle, the autocorrelation should be symmetrical. But in our simulation, some strange phenomena about autocorrelation of segments have been seen. As shown in the Fig. 7 ∼ Fig. 10, results for autocorrelation of 40nm-DNA-minicircle segments are illustrated, with the chemical energy of protein binding increasing, the autocorrelation become not symmetrical, and the right section of the autocorrelation
decreases from 0.8916 to 0.49428, the phenomena mentioned above are caused by protein binding. For worm-like chain model, the autocorrelation can be expressed as $\langle \hat{t}_i \cdot \hat{t}_j \rangle = (\cos \theta)^{j-i}$[8], wherein $\theta$ is the angle between two adjacent segments. For adjacent segments $j - i = 1$ the autocorrelation can be expressed as $\langle \hat{t}_i \cdot \hat{t}_j \rangle = (\cos \theta)^{j-i} = \cos \theta$. In section 3.1, while the chemical potential of protein binding is positive, most the binding sites are occupied by protein, which introduces a binding angle $\pi/3$ between adjacent segments, it is the binding angle between the 39th and 40th segments that makes the right part of the autocorrelation decrease approximating to 0.5 illustrated as Fig. 11, and Fig. 12. For the negative chemical potential, the binding sites on the DNA-minicircle are bonded partially, the angle between adjacent segments is still determined by random conditions, which is so small that the cosine of the binding angle approximate to 1.

3.4 The writhe number distribution

In our simulation, Eq. (5) is used to express the writhe number, as shown in Fig. 15 ~ Fig. 19, the writhe number distribution can be fitted by Gaussian distribution, the length of DNA-minicircle, the chemical potential and the binding angle all affect the writhe number distribution. In Fig. 15, the writhe number distribution

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of 40nm-length minicircle with the chemical potential being 3 fits with the Gaussian distribution very well, no obvious fluctuations are found, at the same time, large writhe number appears in the abscissa, but with the bending angle and length of minicircle increasing, the writhe number distribution fluctuate around the Gaussian function, as shown in Fig. 16, Fig. 17, no large writhe number appears, on the country, the writhe number becomes very small. In Fig. 18, Fig. 19, effects of chemical potential on the writhe number distribution of 40nm-length minicircle with bending angle $\pi/2$, $\pi/3$ respectively are illustrated. Fig. 15 ~ Fig. 19 Effects of preferable angle length of DNA minicircle and chemical potential on the writhe distribution. Fig. 15, Fig. 16 are the writhe number distribution of 40nm-length DNA minicircle with the chemical potential being 3 and the bending angles are $\pi/3$, $\pi/2$ respectively; Fig. 17 is the writhe number distribution of 80nm-length DNA minicircle with the chemical potential being 3 and the bending angles are $\pi/3$; Fig. 18, Fig. 19, the writhe number distribution of 40nm-length DNA minicircle with the bending angles are $\pi/2$, $\pi/3$ respectively changes with chemical potential.
3.5 Characteristics of minicircle under discrete model of DNA-stiffening protein

Above, the occupation fraction, direct conformation, autocorrelation of segments of minicircle and writhe number distribution under the discrete model of DNA-bending protein have been addressed. Wherein all the parameters mentioned above under discrete model of DNA-stiffening protein are discussed in this section, the 80nm-length DNA minicircle under discrete model of DNA-stiffening protein is taken as an example. As shown in Fig. 20 ~ Fig. 22, average occupation number of 80nm-length minicircle without bending angle decreases with chemical potential which ranges from 6.91 to minus 2.3, decreasing from about 46.6 to about 1.8, so chemical potential still have strong effects on the average occupation number. 

Fig. 20 ~ Fig. 22 Average occupation number of 40nm-minicircle changes with chemical potential of protein binding, wherein the discrete model of DNA-stiffening protein is taken, the chemical potential is 6.91, 3 and minus 2.3 respectively. 

The direct conformations of DNA minicircle and the segment autocorrelation under the discrete model of protein-stiffening proteins are illustrated in Fig. 23 ~ Fig. 25 and Fig. 26 ~ Fig. 28. The chemical potential of
protein binding does not have effects on the direct conformations, no folding happens, the direct conformations take on smooth circular as the minicircle of short linear dsDNA should be. Fig. 23 ~ Fig. 25. The direct conformation

conformations of 40nm-minicircle with DNA binding changes with chemical potential of protein binding, wherein the discrete model of DNA-stiffening protein is taken, the chemical potential is 6.91, 3 and minus 2.3 respectively.

The chemical potential decreasing from 6.91 to minus 2.3 does not have obvious effects on the segment autocorrelation, which are all symmetrical. Under the discrete model of stiffening-proteins, the protein-DNA complexes only enhance the bending rigidity of DNA and do not change the bending angle between the adjacent segments, which is still determined by the random conditions, such as thermal excitation, so the angles between adjacent segments are very small, once the protein binds on the dsDNA, the small angles between adjacent segments are fixed at the binding sites, which make the segment autocorrelation in Fig. 26 ~ Fig. 28.

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Fig. 26 ~ Fig. 28. The segment autocorrelation of 40nm-minicircle with DNA binding changes with chemical potential of protein binding, wherein the discrete model of DNA-stiffening protein is taken, the chemical potential is 6.91, 3 and minus 2.3 respectively.

The writhe number distribution in Fig. 29 ~ Fig. 31 still takes on Gaussian distribution, no large writhe number appears under the discrete model of DNA-stiffening protein, which confirms again that the conformations of the DNA minicircle are not folded further and supports the results about the relaxed conformations in Fig. 23 ~ Fig. 25. Fig. 29 ~ Fig. 31. The writhe number distribution of 40nm-minicircle with DNA binding changes with chemical potential of protein binding, wherein the discrete model of DNA-stiffening protein is taken, the chemical potential is 6.91, 3 and minus 2.3 respectively.

References


