Effect of genome sequence on the force-induced unzipping of a DNA molecule

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Abstract

We considered a dsDNA polymer in which distribution of bases are random at the base pair level but ordered at a length of 18 base pairs and calculated its force elongation behaviour in the constant extension ensemble. The unzipping force $F(y)$ vs. extension $y$ is found to have a series of maxima and minima. By changing base pairs at selected places in the molecule we calculated the change in $F(y)$ curve and found that the change in the value of force is of the order of few pN and the range of the effect depending on the temperature, can spread over several base pairs. We have also discussed briefly how to calculate in the constant force ensemble a pause or a jump in the extension-time curve from the knowledge of $F(y)$.

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I. INTRODUCTION

DNA is a giant double stranded linear polymer in which genetic information is stored. Its double stranded helical structure is stabilized by the hydrogen bonding between complimentary bases (A-T are linked with two hydrogen bonds and G-C by three hydrogen bonds) and the stacking interactions of the base pair plateaux. The stacking interactions which impose a well defined distance between the bases and give rise high rigidity to the polymer along its axis depend on the genome sequence. The energy landscape of a dsDNA polymer is therefore expected to depend on the arrangement of bases along the two strands. Knowing this dependence is an important step in understanding the biological functioning of DNA.

With the development of single molecule techniques it has now become possible to probe the force elongation characteristics of a double stranded DNA (dsDNA) polymer and measure its response to an external force or torque in vitro at temperatures where dsDNA is thermally stable. Such measurements give informations about the energy landscape of the molecule. Experiments have usually been performed either in the constant extension or in the constant force ensemble. In the constant extension ensemble the average force of unzipping is found to vary randomly about an average value as the extension is increased, while in the constant force ensemble the unzipped length as a function of time is found to show several pauses and long jumps.

A number of theoretical efforts have recently been made to understand various aspects of dsDNA unzipping. It is shown that while a homogeneous dsDNA gains considerable entropy by opening in response to the external force and therefore the unzipping is entropy driven, a heterogeneous dsDNA is believed to unzip primarily for energetic reasons. Lubensky and Nelson have studied the force induced unzipping of a randomly disordered dsDNA using a Hamiltonian which is coarse grained over many but unknown number of bases. Weeks et al have used the model of Lubensky and Nelson and have calculated the pause point spectrum in the constant force ensemble of a λ phage DNA.

Our aim in this article is to use a Hamiltonian which describes interactions at the base pair level and show that the force-extension curve obtained in the constant extension ensemble provides a more direct exploration of the underlying free energy landscape from the maxima and minima of the force profile. The model described in Sec. 2 also allows us to calculate...
the effect of base pair mutation on the force extension behaviour.

We consider a dsDNA polymer of $N(=Mn)$ base pairs made by repeating $M(M \rightarrow \infty)$ times an oligonucleotide of $n$ base pairs. The oligonucleotide used here to construct the dsDNA polymer has 18 base pairs of which 9 are A-T(or T-A) and other 9 are G-C(or C-G). The arrangement of these base pairs in the oligonucleotide is as shown below;

$$
3' - AGTGACATACTCGACGGA - 5' \\
5' - TCACTGTATGAGCCTGCT - 3'
$$

The dsDNA polymer constructed in this way is heterogeneous as it contains both A-T and G-C base pairs. However, because of the repetition of the oligonucleotide the distributions of bases in the dsDNA polymer are not random but have a periodicity at the length of the oligonucleotide. We therefore expect its properties to lie in between a homogeneous and a randomly disordered dsDNA polymer.

II. THE MODEL AND ITS THERMODYNAMICS

We represent the interactions in the polymer at the base pair level by the model Hamiltonian of Peyrard and Bishop (PB) which though ignores the helicoidal structure of the dsDNA polymer, has enough details to analyze mechanical behaviour at few Å scale relevant to molecular-biological events and can easily be extended to include the effect of heterogeneity in the base pair sequence. The PB model for a heterogeneous DNA polymer is written as,

$$
H = \sum_i \left[ \frac{p_i^2}{2m} + V_i(y_i) + W(y_i, y_{i+1}) \right]
$$

(1)

where $m$ is the reduced mass of a base pair (taken to be same for both A-T and G-C base pairs and equal to 300 a.m.u.), $y_i$ denotes the stretching of the hydrogen bonds connecting the two bases of the $i^{th}$ pair and $p_i = m(dy_i/dt)$. The on-site potential $V_i(y_i)$ which describes interactions of two bases of the $i^{th}$ pair is represented by the Morse potential

$$
V_i(y_i) = D_i [e^{-a_i y_i} - 1]^2
$$

(2)

where $D_i$ measures the depth of the potential and $a_i$ its range. Both $D_i$ and $a_i$ depend on whether the $i^{th}$ base pair is A-T or G-C. The stacking interaction term of the PB model
TABLE I: Value of $\Delta \rho_{i,i+1}$ for all possible combination of two consecutive base pairs. Two consecutive bases along a strand is shown. Other two bases of the quartet are complementary to these. These values are found using the data of stacking energy of Sponer et al [18] and taking $\bar{\rho} = 5.0$.

| Base Quartet (3’-5’) | $\Delta \rho_{i,i+1}$ | Base Quartet (3’-5’) | $\Delta \rho_{i,i+1}$ |
|----------------------|-----------------------|-----------------------|-----------------------|
| AA                   | -0.10                 | GA                    | -0.10                 |
| AT                   | -0.77                 | GT                    | -0.14                 |
| AG                   | -0.53                 | GG                    | -0.34                 |
| AC                   | -0.14                 | GC                    | +2.39                 |
| TA                   | -0.53                 | CA                    | +0.36                 |
| TT                   | -0.10                 | CT                    | -0.53                 |
| TG                   | +0.36                 | CG                    | +0.48                 |
| TC                   | -0.10                 | CC                    | -0.34                 |

is modified and is written as

$$W(y_i, y_{i+1}) = \frac{1}{2} k \left[ 1 + \rho_{i,i+1} e^{-b(y_i+y_{i+1})} \right] (y_i - y_{i+1})^2$$ (3)

where force parameter $k$ measures the stiffness of a single strand of the molecule and the second term in the bracket represents the anharmonic term. The strength of anharmonic term is measured by $\rho_{i,i+1}$ and its range by $b$. We allow the value of the parameter $\rho_{i,i+1} (= \bar{\rho} + \Delta \rho_{i,i+1})$ to depend on the arrangement of four bases in the consecutive base pairs $i$ and $(i+1)$. In our calculation we have taken $D_{AT} = 0.058$ eV, $D_{GC} = 0.087$ eV, $a_{AT} = 4.2$ Å$^{-1}$, $a_{GC} = 6.3$ Å$^{-1}$, $b = 0.35$ Å$^{-1}$, $k = 0.02$ eVÅ$^{-2}$, $\bar{\rho} = 5.0$ and used the data of stacking energies given by Sponer et al [18] to estimate the value of $\Delta \rho_{i,i+1}$. We list in Table 1 the value of $\Delta \rho_{i,i+1}$ for all possible combinations of two consecutive base pairs. We treat the nucleotide the repetition of which forms the dsDNA polymers as an effective base pair and define its kernel as,

$$\bar{K}(y_1, y_n) = \int dy_2, ... dy_{n-1} \ K(y_1, y_2), ...$$

$$\times K(y_i, y_{i+1}), ... K(y_{n-1}, y_n)$$ (4)
and

\[ H(y_i, y_{i+1}) = \frac{1}{2} [V(y_i) + V(y_{i+1})] + W(y_i, y_{i+1}) \]

where \[ K(y_i, y_{i+1}) = \left( \frac{\beta k^2}{2\pi} \right)^{1/2} \exp\left[ -\beta H(y_i, y_{i+1}) \right] \] (5)

Equation (4) is evaluated by the method of matrix multiplication. For this we chose -5.0 Å and 200.0 Å, respectively, as the lower and upper limits of integration for each variable and discretized the space using the Gaussian quadrature with number of grid points equal to 900. The resulting matrix \( \hat{K}(y_1, y_n) \) is a 900 × 900 square matrix.

The configurational partition function \( Z_N^c \) defined as

\[ Z_N^c = \int \prod_{p=1}^{M} dy_p K(y_p, y_{p+1}) \delta(y_1 - y_{N+1}) \] (6)

has been evaluated by the matrix multiplication method for several values of \( N \) ranging between 3000 to 6000. As all matrices in Eq.(6) are identical the multiplication is done very efficiently. The resulting partition function is used to calculate the free energy per base pair from the following relation

\[ f = -\frac{1}{2} k_B T \ln \left( \frac{4\pi^2 k_B T^2 m}{k} \right) - \frac{k_B T}{N} \ln Z_N^c \] (7)

where the first term on the r.h.s. is due to the kinetic energy. We found that for \( N \geq 4000 \) the value of \( f \) is independent of the value of \( N \) taken in the calculation of \( Z_N^c \).

We have also diagonalized the matrix \( \hat{K}(y_p, y_{p+1}) \) to find first two eigenvalues \( \lambda_0 \) and \( \lambda_1 \) where \( \lambda_0 = \exp(-\beta E_0) \) and \( \lambda_1 = \exp(-\beta E_1) \), using a method described in ref [19]. Since \( E_0 \) and \( E_1 \) are the eigenenergies of a kernel having \( n = 18 \) base pairs, the average eigenenergies per base pair is \( \epsilon_i = E_i/n \). In Fig. 1(a) we plot the eigenenergies \( \epsilon_0 \) and \( \epsilon_1 \). We find that \( \Delta \epsilon(T) = \epsilon_0 - \epsilon_1 \propto (T_D - T)^\nu \) with \( \nu = 1 \) and \( T_D = 356.7 \) K. The behaviour of \( \Delta \epsilon(T) \) as a function of \( T \) is found to be same as in the case of a homogeneous dsDNA polymer [14]. In the thermodynamic limit the value of configurational partition function \( Z_N^c \) is determined by \( \lambda_0 \) and therefore \( Z_N^c = \lambda_0^N = \exp(-\beta N \epsilon_0) \). The free energy per base pair calculated using this value of \( Z_N^c \) agrees very well with the values found from Eq.(7).

The value of \( f \) as a function of temperature \( T \) is shown in Fig. 1(b). A cusp in \( f \) at the thermal denaturation temperature \( T_D = 356.7 \) K is clearly seen. The existence of cusp
FIG. 1: (a) The average per base pair eigenenergies $\epsilon_0$ and $\epsilon_1$ of the kernel of Eq.(4) as a function of temperature are shown. (b) The free energy per base pair as a function of temperature is shown. A cusp in the free energy is seen at the thermal denaturation temperature $T = T_D = 356.7$ K where $\Delta \epsilon = \epsilon_0 - \epsilon_1 \sim 0$.

indicates that the thermal denaturation transition is first order with a sudden jump in its entropy.

Next we calculate the unzipping of the polymer in the constant extension ensemble in which separation of one ends of the two strands of the molecule is kept fixed and the average force needed to keep this separation is measured. The work done in stretching the base pair 1 to $y$ distance apart is

$$W(y) = \frac{1}{2} V_1(y) - k_B T [\ln Z_c^c(y) - \ln Z_c^c]$$

where

$$Z_c^c(y) = \int \prod_{p=1}^M dy_p \delta(y_1 - y) \delta(y_N - 0) K(y_p, y_{p+1})$$
and $Z_N^N$ is defined by Eq.(6). The force $F(y)$ as a function of extension $y$ is found from the relation,
\[
F(y) = \frac{\partial W(y)}{\partial y}
\]  
\(10\)

III. RESULTS AND DISCUSSIONS

In Fig. 2 we plot the value of $F(y)$ as a function of extension $y$ for $T = 100$ K and 300 K. To show the height and width of peak in $F(y)$ at small values of $y$ as well as oscillations at larger values of $y$ we chose different scales on the two sides of $y = 5.0$ Å. Though the experiments are generally done at temperatures close to 300 K, the motivation for studying the behaviour of dsDNA at 100 K is to illustrate the effect of temperature on the energy landscape. Figure 2 shows a large force barrier at $y \sim 0.2$ Å, a feature similar to that found in the case of a homogeneous dsDNA polymer \[13, 14\]. The height of this peak is nearly 230 pN at 100 K and 215 pN at 300 K. The physical origin of this barrier is in the potential well due to hydrogen bonding plus the additional barrier associated with the reduction in DNA strand rigidity as one passes from dsDNA to ssDNA. Since the peak corresponds to a process in which only one or two base pairs participate the effect of thermal energy in formation of the barrier is small.

For $y \geq 1.0$ Å we, however, find that the two curves of $F(y)$ considerably differ from each other as well as from that of a homogeneous DNA polymer. While in case of a homopolymer the peak in $F(y)$ decays as $y$ is increased and for $y \geq 10.0$ Å attains a constant value equal to that of the critical force found from the constant force ensemble to unzip the dsDNA into two ssDNA \[14\], here we find that $F(y)$ curve oscillates about a mean value. These oscillations are due to maxima and minima in the energy landscape and these maxima and minima depend on the genome sequence in the two strands of the DNA polymer. It is easy to realize that a G-C rich region of the polymer has energy minimum whereas the A-T rich region has energy maximum. Therefore, when the front of the unzipping fork enters the G-C rich region it needs larger force to come out of it whereas in case of the A-T rich region it needs less force than the average to move forward. As is evident from Fig. 2, the maxima and minima in the energy landscape are much more pronounced at 100 K compared to that at 300 K; the thermal fluctuations have tendency to suppress the local variation and make the energy landscape relatively smooth.
FIG. 2: The average force $F(y)$ in pN at $T = 100$ K and 300 K required to stretch one of the ends base pair to a distance $y$ is shown. Different scales are chosen for the two sides of $y = 5.0$ Å.

At $T = 300$ K the oscillations in $F(y)$ are found to decay (see Fig. 2) rather rapidly and for extension greater than 100 Å the dsDNA polymer seems to behave like a homopolymer in which unzipping takes place continuously at constant rate when the applied force exceeds the critical force. But at $T = 100$ K the oscillations in $F(y)$ persists for much larger values of $y$. As the extension $y$ increases, however, the wiggles in $F(y)$ get smoothened and the peak heights decrease though very slowly. These features arise due to the contribution made to the free energy by the fluctuations of the single stranded part of the unzipping fork. As $y$ increases the single stranded part increases resulting in larger entropic contributions to the free energy and thus reducing the barrier encountered by the front of the fork. After certain length of the ssDNA part the entropic contribution to free energy per base pair gets saturated and oscillations in $F(y)$ if not already suppressed will remain unaffected on further increasing the extension. Therefore the effect of genome sequence on the unzipping depends on the depth of the local energy minimum. In the case of the dsDNA polymer considered here the variations in the energy along the polymer gets averaged out at 300 K and therefore the unzipping beyond about 100 base pairs becomes similar to that of a homopolymer. But at 100 K the local minimum in the energy landscape are deep enough to show variations in the unzipping force for large enough extensions.
FIG. 3: The change in $F(y)$ when a base pair C-G at position 10 is changed by a base pair T-A (dotted line) and when a base pair A-T at position 15 is changed by a base pair G-C (dashed line) is shown at temperatures 100 K and 300 K. The full line corresponds to the original dsDNA polymer. This change is due to the combined effect of the change in the on-site and the stacking potentials.

To examine more closely the sensitivity of the force - extension curve on the genome sequence we altered base pairs on selected positions and calculated their effects on the $F(y)$ curve. We indicate a base pair by its number counted from the stretched end taking the base pair that is being stretched as 1. First we alter a base pair at one position and calculate its effect. In Fig. 3 we show our results for two cases, (i) a base pair C-G at position 10 is replaced by a base pair T-A (shown by dotted line) and (ii) a base pair A-T at position 15 is replaced by a base pair G-C (shown by dashed line). These replacements have brought changes in both the on-site potential $V(y)$ and in the stacking interactions. The change in the stacking interactions is measured by the change in $\Delta \rho$ which for base pairs 9-10 has changed from -0.14 to -0.77 and for the base pairs 10-11 from -0.53 to -0.10. When the base pair at position 15 has been changed from A-T to G-C the value of $\Delta \rho$ has changed for base pairs 14-15 from -0.10 to -0.34 and for base pair 15-16 from -0.14 to +2.39. The
FIG. 4: The change in the value of $F(y)$ when a base pair in each repeating nucleotide is introduced at $T = 100$ K and 300 K. The dotted line corresponds to the change introduced at positions 10, 28, 46, ... by replacing C-G base pair by T-A base pair. Similarly the dashed line corresponds to the situation when the base pair A-T from positions 15, 33, 51, ... is replaced by the G-C base pair. The full line corresponds to the original dsDNA polymer.

change in the $F(y)$ curve brought by the change in the base pair sequence is therefore due to the combined effect of the change in the on-site and the stacking potentials. From Fig. 3 we note that the change in $F(y)$ value is of the order of 5-7 pN and the range of the effect spreads about 14-17 Å. This means that while the effect is localized to about 7-8 base pairs at 100 K, at 300 K it spreads to almost the length of the oligonucleotide.

In Fig. 4 we compare the results found by replacing a base pair in each repeating nucleotide at the same position, i.e. a periodic change in the base pair sequence with the length of periodicity equal to that of the oligonucleotide. For example, the change introduced at positions 10 now repeats along the polymer at positions 10, 28, 46, 64,... and the change made at position 15 now repeats along the polymer at positions 15, 33, 51, 69,... While the change introduced in the $F(y)$ curve due to this change in genome sequence follow the periodicity of the change at 100 K, at 300 K the entire curve either moves up or down by
about 1 pN for \( y \geq 50 \text{ Å} \).

IV. CONCLUSIONS

We have modified the stacking energy part of the PB Hamiltonian so that the effect arising due to genome sequence in a dsDNA molecule is fully accounted for. Using the method of matrix multiplications we have done essentially exact thermodynamics of this Hamiltonian in the constant extension ensemble. The results given above suggest that the genome sequence has a very specific effect on the unzipping of a dsDNA polymer and can therefore be used to find this sequence by determining the force extension curve in the constant extension ensemble. Any change in this sequence can change the force-extension curve along the length of few base pairs. Such a change in unzipping process may have an important effect on the DNA transcription and replication dynamics.

The above results can also be used to calculate the unzipping properties of the molecule in the constant force ensemble. For example, the constant force ensemble partition function can be found from the relation,

\[
Z_N^c(F) = \int dy \; Z_N^c(y) \; e^{\beta F y} = Z_N^c \int dy \; e^{\beta (F y - W(y))}
\]

and the time needed to cross a force peak (or valley) encountered by the front of the unzipping fork from the relation

\[
t = t_0 \exp \left( \beta \int_{y_1}^{y_2} dy' (F(y') - F) \right)
\]

where \( F(y_1) - F = F(y_2) - F = 0 \) and \( t_0 \) is time needed by the front to move the distance; \( \Delta y = y_2 - y_1 \) under the influence of the force \( F \) when there is no peak or valley. A natural extension of the method developed here is to apply it to estimate the force-elongation behaviour of a natural DNA [20].

Experimental results both in the constant extension and in the constant force ensemble are available for lambda phage DNA which is 48,502 base pairs long. This DNA is particularly interesting as it consists of a GC rich half connected to an AT rich half and therefore expected to have different energy landscape viewed from the two ends. Though for quantitative comparisons between the experimental results and the theory one has to consider the genome sequence of the DNA from the end used in the experiment, the qualitative features of the experimental results are in agreement with the features of the force-extension curve discussed
above. It may also be pointed out that while the method described above give the force behaviour at the base pair level the results found experimentally are still at a level of several base pairs.

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