Statistical Theory of Force Induced Unzipping of DNA

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Abstract

The unzipping transition under the influence of external force of a dsDNA molecule has been studied using the Peyrard-Bishop Hamiltonian. The critical force \( F_c(T) \) for unzipping calculated in the constant force ensemble is found to depend on the potential parameter \( k \) which measures the stiffness associated with a single strand of DNA and on \( D \), the well depth of the on-site potential representing the strength of hydrogen bonds in a base pair. The dependence on temperature of \( F_c(T) \) is found to be \( (T_D - T)^{1/2} \) (\( T_D \) being the thermal denaturation temperature) with \( F_c(T_D) = 0 \) and \( F_c(0) = \sqrt{2kD} \). We used the constant extension ensemble to calculate the average force \( F(y) \) required to stretch a base pair \( y \) distance apart. The value of \( F(y) \) needed to stretch a base pair located far away from the ends of a dsDNA molecule is found twice the value of the force needed to stretch a base pair located at one of the ends to the same distance for \( y \geq 1.0 \) Å. The force \( F(y) \) in both cases is found to have a very large value for \( y \approx 0.2 \) Å compared to the critical force found from the constant force ensemble to which \( F(y) \) approaches for large values of \( y \). It is shown that the value of \( F(y) \) at the peak depends on the value of \( k\rho \) which measures the energy barrier associated with the reduction in DNA strand rigidity as one passes from dsDNA to ssDNA and on the value of the depth of the on-site potential. The effect of defects on the position and height of the peak in \( F(y) \) curve is investigated by replacing some of the base pairs including the one being stretched by defect base pairs. The formation and behaviour of a loop of Y shape when one of the ends base pair is stretched and a bubble of ssDNA with the shape of “an eye” when a base pair far from ends is stretched are investigated.

PACS numbers: 87.14.Gg, 87.15.Aa, 64.70.-p

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I. INTRODUCTION

Natural DNA is a giant double stranded linear molecule with length ranging from $2\mu m$ for simple viruses to $3.5 \times 10^7 \mu m$ for more complex organisms and is known to have a complex nature of internal motions [1]. The structural elements such as individual atoms, group of atoms (bases, sugar rings, phosphates) fragments of double chain including several base pairs, are in constant movements and this movement plays crucial role in its biological activities. During transcription a transient "bubble" of single stranded DNA is formed, to allow enzymes that make a mRNA copy of DNA sequence to access the DNA bases [2]. In a replication the separation of two strands starts from one end and propagates to the other end; separated parts of each strand serves as a template for the synthesis of a new strand and thus making two exact copies of the DNA. The energy involved in these processes is of the order of 5-25 kcal/mole. These motions in vitro can be activated by increasing temperature, changing pH and/or solvent conditions. The process of separating the two strands wound in a double helix into two single strands upon heating is known as thermal denaturation. Several experiments on dilute DNA solutions [3] have provided evidence for the existence of a thermally driven melting transition corresponding to the sudden opening of base pairs at a denaturation or melting temperature $T_D$.

In the living organisms the DNA strands are forced open by proteins which pull the strands of DNA on selected positions. The recently developed experimental techniques of micromanipulations and nanomanipulations [4, 5, 6] have now made it possible to probe the force elongation characteristics of double stranded DNA molecule (dsDNA), determining its response to external force and torque in vitro at temperatures where dsDNA is thermally stable in absence of the external force. The mechanical unzipping of dsDNA by a force pulling the end of one of the two strands, the end of the other strand being anchored to some physical support (see Fig. 1) has been studied by Bockelmann and co-workers [7] who measured the average force for the opening of the two strands. It has been shown that the two strands of a dsDNA can be pulled apart if a force $\approx 12\ pN$ is applied with some variation about this mean value depending upon the sequence. In single molecule experiments, the results may depend on the statistical ensemble one works with [8, 9]. It may be noted that techniques like atomic force microscope [10] use the fixed extension ensemble while the magnetic bead method [11] uses the fixed force ensemble. In view of this we have studied...
the unzipping transitions in both the fixed force (sec. 4) and the fixed extension (sec. 5) ensembles.

In order to make a theoretical approach feasible one has to reduce the complexity of internal motions of dsDNA to the minimum. Clearly, an appropriate choice of the relevant degrees of freedom depends on the problem one is interested. For example, the models based on the theory of polymers that use self-avoiding walks to describe the two strands \[12\] can be very successful in studying the properties of melting transition at the large scale but they cannot be used to investigate the properties that depend on the sequence, or probe the DNA at the microscopic scale as is done in some single molecule experiments. One of the simplest models that investigate DNA at the scale of base pairs is the Peyrard-Bishop model \[13, 14\]. Though as described in Sec. 2, this model ignores the helicoidal structure \[15\] of DNA, it is found to have enough details to analyze mechanical behaviour at the few Å scale relevant to molecular-biological events.

A number of attempts have recently been made to understand various aspects of dsDNA unzipping \[8, 9, 16, 17, 18, 19, 20\]. Our aim in this work is to give further insight on the various intricacies involved in the unzipping of dsDNA. In particular, we investigate
how the unzipping transition depends on the different parameters that appear in the model Hamiltonian and on the presence of defect base pairs. The paper is organized as follows: In Sec. 2 we describe in detail the different parts of the Peyrard-Bishop (PB) model and the set of potential parameters that we use in our study. In Sec. 3 we provide a brief outline of the theory that is used to investigate the transitions in a homogeneous dsDNA. This theory is used in Sec. IV to calculate the value of critical force, $F_c(T)$, for unzipping as a function of temperature. The curve $F_c(T)$ gives the boundary separating the zipped state from the unzipped state. The critical force is shown to depend most predominantly on the stiffness associated with a single strand. In Sec. 5 we investigate the unzipping of dsDNA in a constant extension ensemble and calculate the average force needed to maintain the extension. It is shown that a very large force is needed to enforce an extension of about 1Å and the value of this force depends on the barrier associated with reduction in DNA strand rigidity as one passes from dsDNA to ssDNA. The change in height and position of the barrier is investigated by introducing defect base pairs. The extension can take place at any point along the strand. We have discussed the two cases; the extension at one end and at the middle. We summarize our results in Sec. 6.

II. MODEL HAMILTONIAN AND POTENTIAL PARAMETERS

Since the internal motion that is predominantly responsible for unzipping of dsDNA in situation shown in Figure 1 is the stretching of the bases from their equilibrium position along the direction of the hydrogen bonds that connect the two bases, a DNA molecule can be considered as a quasi one dimensional lattice composed of $N$ base pair units. The forces which stabilize the structure are the hydrogen bonds between complementary bases on opposite strands and stacking interactions between nearest neighbour bases on opposite strands. Each base pair is in one of the two states: either open (non hydrogen bonded) or zipped (hydrogen bonded). In the presence of a force acting on one end of the base pair, the Hamiltonian can be written as,

$$H = \sum_{n=1}^{N} H(y_n, y_{n+1}) - F \cdot y$$  \hspace{1cm} (1)

where $y_n$ denotes the stretching from the equilibrium position of the hydrogen bonds connecting the two bases of the $n^{th}$ pair. A model Hamiltonian that contains the minimum
complexity of the internal motion at base pairs level and accounts for the stability of ds-DNA structure is \[13\].

\[ H(y_n, y_{n+1}) = \frac{p_n^2}{2m} + w(y_n, y_{n+1}) + V(y_n) \]  

(2)

where \( m \) is the reduced mass of a base pair, and

\[ p_n = m \left( \frac{dy_n}{dt} \right) \]  

(3)

The on-site potential \( V(y_n) \) describes the interaction of the two bases of the \( n^{th} \) pair. The Morse potential

\[ V(y_n) = D_n(e^{-ay_n} - 1)^2 \]  

(4)

is generally taken to represent the on-site interaction. It may be noted that \( V(y_n) \) does not represent only the hydrogen bonds connecting two bases belonging to opposite strands, but also the surrounding solvent effects and the repulsion interactions of the phosphates. In Eq. (4) \( D_n \) measures the depth of the potential and \( a \) its range. In a homogeneous DNA, \( D_n \) is taken to be site independent but in a heterogeneous (or natural) DNA the value of \( D_n \) depends on whether the \( n^{th} \) base pair is AT or CG. The flat part at large values of the displacement of this potential emulates the tendency of the pair “melt” at high temperatures as thermal phonons drive the molecule outside the well and towards the flat portion of the potential.

The stacking interactions are contributed by dipole-dipole interactions, \( \pi \)-electron systems, London dispersion forces and in water solution, the solvent induced hydrophobic interactions. These forces result in a complex interaction pattern between overlapping base pairs, with minimum energy distance close to 3.4 Å in the normal DNA double helix. As suggested by Peyrard and Bishop [14], the following anharmonic potential model mimic the essential features of the stacking energy:

\[ w(y_n, y_{n+1}) = \frac{1}{2}k \left[ 1 + \rho e^{-b(y_n+y_{n+1})} \right] (y_n - y_{n+1})^2 \]  

(5)

where the force constant \( k \) is related with the stiffness of a single strand and the second term in the bracket represents the anharmonic term. The ”anharmonic range” is defined by the parameter \( b \). In the zipped state the force constant is equal to \( k(1 + \rho) \). Decrease in the force constant in the unzipped state provides a large entropy and hence favours unzipping either at high force or at high temperatures. The difference in the force constants between
the zipped and the unzipped state of base pairs, creates an energy barrier the value of which depends on $\rho$ and range $b$.

The Hamiltonian model described above has five parameters $D_n$, $k$, $\rho$, $a$ and $b$. In our calculation described below we have taken $a = 4.2$ Å$^{-1}$ and $b = 0.35$ Å$^{-1}$. For other three parameters two different sets of values have been used; (i) $D_n = 0.11$ eV, $\rho = 475.0$, $k = 0.0032$ eVÅ$^{-2}$ and (ii) $D_n = 0.063$ eV, $\rho = 5.0$, $k = 0.025$ eVÅ$^{-2}$. We shall henceforth refer them as potential parameters of set (i) or (ii). The values of set (i) are close to those taken by Cocco et al [19] to study the unzipping of DNA at $T = 298$ K under the influence of force, whereas those of set (ii) are close to those used by us [21] and by others [14, 22, 23] in the study of thermal denaturation of DNA. For the reduced mass $m$, a value of 300 a.m.u. has been used. For both sets of parameters the denaturation temperature in absence of force is found close to 350 K (see below) which, in turn is close to the value found for a natural DNA. The denaturation transition is found to be first order for both set of parameters with finite melting entropy, a discontinuity in the fraction of bound base pairs and divergent correlation lengths.

III. STATISTICAL THEORY FOR UNZIPPING TRANSITION

We consider a dsDNA having $N$ number of base pairs at temperature $T$. The classical partition function of it can be written as

$$Z_N(\beta) = Z_N^c(\beta)Z_N^k \left(\frac{\beta k}{2\pi}\right)^{-N/2}$$

where

$$Z_N^k = (2\pi mk_BT)^{N/2}$$

is the kinetic part. The configurational part of the partition function is written as (taking $y_N$ to be fixed at zero value)

$$Z_N^c = \left(\frac{\beta k}{2\pi}\right)^{N/2} \int \left(\prod_{n=1}^{N} dy_n\right) \delta(y_N - 0)$$

$$\times \exp\left\{-\beta \sum_{n=1}^{N-1} \frac{1}{2}(V(y_n) + V(y_{n+1})) + w(y_n, y_{n+1}) - F(y_n - y_{n+1})\right\}$$

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where \( F \) is the force. Note that the factor \((\beta k/2\pi)^{-N/2}\) included in Eq. (6) is balanced by its inverse included in Eq. (8). For \( N \to \infty \) and force, \( F \), acting on \( n = 1 \) base pair, taking \( y_N = 0 \) is justified. In denaturation study of DNA when \( F = 0 \) and the ends are free, the periodic boundary condition, which amounts to adding a fictitious base pair with index \( N + 1 \) having the same dynamics as base pair 1 (i.e. \( y_{N+1} = y_1 \)), is imposed.

In absence of force \((F = 0)\) and for a homogeneous chain a direct calculation of the partition function \( Z_c^N \) can be performed by the transfer integral (TI) method \[24, 25\]. For this it is enough to know the eigenvalues of the TI;

\[
a \left( \frac{\beta k}{2\pi a^2} \right)^{1/2} \int dy' \exp \left\{ -\beta \left[ \frac{1}{2}(V(y) + V(y')) + w(y, y') \right] \right\} \times \phi_i(y') = e^{-\beta \epsilon_i} \phi_i(y)
\]

The properties and numerical methods for evaluating the eigenvalues and eigenfunctions from Eq.(9) have been discussed in details by Dauxois and Peyrard \[24\] and Zhang et al \[25\]. We have chosen -5.0 Å and 195.0 Å as lower and upper limits of integration and use the Gauss-Legendre method to discretize the integral and choose the number of points \( M = 900 \). The eigenvalues and eigenfunctions are found by diagonalizing the resulting matrix. The values of the two lowest eigenvalues \( \epsilon_0 \) and \( \epsilon_1 \) are plotted in Fig. 2 as a function of temperature for the two sets of potential parameters. At the thermal denaturation temperature \( T_D \), \( \Delta \epsilon = \epsilon_1 - \epsilon_0 \sim 0 \) is minimum. We found that in both cases \( \Delta \epsilon \sim 10^{-5} \) at \( T_D \). The value of \( T_D \) found for the potential parameters of set (i) is 350.28 K and for the potential parameters of set (ii) 349.66 K. It seems that the melting temperature depends on the collective effect of different parts of interaction rather than on any one of them.

The order of the transition is determined by the exponent \( \nu \) which characterize the gap \( \Delta \epsilon \propto (T_D - T)^\nu \) at temperature \( T \leq T_D \); a value \( \nu = 1 \) implies a cusp in the free energy and a discontinuous entropy, a feature of first order transition, whereas a value equal to 2 corresponds to 2nd order transition. Our results plotted in figures (See Fig. 2) show \( \nu = 1 \) for both cases. The free energy per base pair is determined by (see Eq.(6))

\[
f = -\frac{1}{2} k_B T \ln \left( \frac{4\pi^2 k_B T^2 m}{k} \right) + \epsilon_0
\]

The thermodynamic quantities like the entropy \( s \), the specific heat \( c_v \) are evaluated from the standard relations,

\[
s = -\frac{\partial f}{\partial T}; \quad c_v = -T \frac{\partial^2 f}{\partial T^2}
\]
FIG. 2: The two lowest eigenvalues $\epsilon_0$ and $\epsilon_1$ of TI (Eq. (9)) are plotted as a function of temperature for potential parameters of set (i) and set (ii). The temperature at which $\Delta \epsilon = \epsilon_1 - \epsilon_0$ becomes zero is the denaturation temperature $T_D$. The value of $T_D$ for the two sets of potential parameters is also shown in the figure.

A cusp in $f$ at the thermal denaturation temperature $T_D$ is distinctly seen in the plot of $f$ vs. $T$ in Fig. 3. The cusp lies at the point where $\Delta \epsilon = \epsilon_1 - \epsilon_0$ becomes zero, i.e. at $T = T_D$. A sharp jump in entropy occurs at $T = T_D$. The value of the jump are found to be $\Delta s = 3.90$ $k_B$ and 2.73 $k_B$, respectively, for potential parameters of set (i) and (ii).
This result as well as the equilibrium value of base pair stretching $\langle y \rangle$ calculated from the relation

$$\langle y \rangle = \int_{-\infty}^{\infty} y |\phi_0(y)|^2 dy$$

(12)

where $\phi_0(y)$ is the eigenfunction associated with $\epsilon_0$ suggest that the denaturation transition is first order for both sets of parameters; though the possibility of underlying continuous transition cannot be ruled out [26]. The apparent first order transition has its origin in the fact that the thermally generated barrier has a sufficiently larger range than the Morse potential. A crossover from first order to second order transition is found for $b/a > 0.5$ [23].

The TI of Eq.(9) can be reduced to a one dimensional Schrödinger equation [19, 23]

$$\left[ -\frac{(k_BT)^2}{2kg(y)} \frac{\partial^2}{\partial y^2} + U(y) \right] \phi_i(y) = \epsilon_i \phi_i(y)$$

(13)

where $g(y) = 1 + \rho e^{-2bg}$ and $U(y) = V(y) + (1/2)k_BT \ln g(y)$.

The solution of Eq.(13) is exactly known when $g(y)$ is replaced by $g(0)$. For the ground (zipped) state this is a reasonable approximation. Thus we have [27]

$$\epsilon_0 = ak_BT \sqrt{\frac{D}{2kg(0)}} - \frac{(ak_BT)^2}{8kg(0)} + \frac{1}{2}k_BT \ln g(0)$$

(14)

The value of $\epsilon_0$ found from this relation is close to the one calculated numerically from Eq.(9). The ground state wave function $\phi_0(y)$ for $y \gg 1/a$ (outside the Morse potential well) can be expressed as

$$\phi_0(y) = \frac{A}{\sqrt{p(y)}} \exp \left[ -\int_{y_0}^{y} dy' \sqrt{p(y')} \right]$$

(15)

where $y_0$ is found from the relation

$$U(y_0) = \epsilon_0$$

and

$$p(y) = \frac{1}{k_BT} \sqrt{2kg(y)(U(y) - \epsilon_0)}$$

As in the WKB approximation, the coefficient $A$ can be found by connecting the expression of $\phi_0(y)$ from the two regions in such a way that both the wave function and its derivative are continuous functions of $y$. 

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FIG. 3: The free energy evaluated using Eq.(10) is plotted as a function of temperature for potential parameters of set (i) and set (ii). A cusp in free energy at temperature $T = T_D$ is clearly seen.

IV. FORCE INDUCED UNZIPPING: CONSTANT FORCE ENSEMBLE

When a force $F$ is applied on one end of the dsDNA as shown in Fig. 1, it favours the separation of the two strands and one expects a critical force $F_c(T)$ for unzipping. At a given temperature, $T$, when the applied force $F$ is less than $F_c(T)$ the DNA remains in the zipped state and is described by the lowest eigenvalue $\epsilon_0$ and eigenfunction $\phi_0(y)$ of Eq.(9).
But for $F > F_c(T)$ the two strands of DNA will get separated. The free energy per base pair of unzipped DNA (i.e. when the particle moves on the plateau of the Morse potential) can easily be calculated from Eq.(8) as the Hamiltonian in this case reduces to

$$H(y_n, y_{n+1}) = D + \frac{1}{2} k (y_n - y_{n+1})^2$$  \hspace{1cm} (16)

Denoting the free energy per base pair of the unzipped DNA chain by $g_u$, we get from Eqs.(8) and (16),

$$g_u = - \frac{k_B T}{N} \ln Z_N = D - \frac{F^2}{2k}$$  \hspace{1cm} (17)

The transition from the zipped to unzipped state takes place when $g_u$ becomes equal to $\epsilon_0$. Thus the critical force needed to unzip the dsDNA chain at temperature $T$ is found to be

$$F_c(T) = \sqrt{2k(D - \epsilon_0)}$$  \hspace{1cm} (18)

The expression for $F_c(T)$ given by Eq.(18) can also be found using the following procedure. When the unzipping force $F$ is included in the expression of the TI of Eq.(9) the one dimensional Schrödinger equation becomes \[19,\]

$$\left[ - \frac{(k_B T)^2}{2kg(y)} \frac{\partial^2}{\partial y^2} + \frac{F(k_B T)}{kg(y)} \frac{\partial}{\partial y} + U(y) - \frac{F^2}{2kg(y)} \right] \psi_i(y) = \epsilon_i \psi_i(y)$$  \hspace{1cm} (19)

If we substitute the transformation

$$\psi(y) = e^{Fy/k_BT} \phi(y)$$  \hspace{1cm} (20)

in Eq.(19) it reduces to Eq.(13). Equation(20) suggests that the force $F$ biases the eigenfunction in the direction of the force. However, as argued by Lubensky and Nelson \[9\] the transformation of Eq.(20) is valid as long as the eigenfunction $\psi(y)$ satisfies the same boundary conditions as the eigenfunction $\phi(y)$ and for $\psi_0(y)$ to be well behaved it is essential that $\phi_0(y)$ should decay at large values of $y$ at least as fast as $\exp(-Fy/k_BT)$.

From Eq.(15) we know that $\phi_0(y)$ decays at large values of $y$ (note that at large $y$, $g(y) = 1$ and $V(y) = D$) as

$$\phi_0(y) \sim \exp \left[ - \frac{y}{k_BT} \sqrt{2k(D - \epsilon_0)} \right]$$  \hspace{1cm} (21)

The value of force at which the transformation of Eq.(20) breaks down corresponds to the critical force. Thus from Eqs.(20) and (21) one gets the expression for $F_c(T)$ given by Eq.(18).
FIG. 4: Variation of critical force $F_c(T)$ as a function of temperature for the two sets of potential parameters. The dashed line corresponds to potential parameters of set (i) and full line to set (ii).

The curve $F_c(T)$ plotted in Fig. 4 gives the boundary that separates the zipped state of DNA from the unzipped state in $(T, F)$ plane. We may note that the value of $F_c(T)$ for the set of potential parameters (i) is less than that of the force parameters of set (ii) and this difference increases with $(T_D - T)$. This behaviour can easily be understood from the fact that the value of $F_c(T)$ depends on the product of $k(D - \epsilon_0)$. Since $D - \epsilon_0(T)$ is proportional to $T$ having values equal to $D$ at $T = 0$ K and zero at $T = T_D$, we have

$$F_c(T) = \sqrt{2kD}\left(1 - \frac{T}{T_D}\right)^{1/2} \tag{22}$$

At a given $T$ the value of $F_c(T)$ depends on $\sqrt{kD}$ as for both sets of parameters $T_D$ are nearly equal. This explains the difference in the value of $F_c(T)$ found for the two sets of parameters. Equation (22) is important as it gives the dependence of $F_c(T)$ on the stiffness of single strand, the potential well depth of interaction in a base pair and on the temperature.

It may, however, be noted that the experimental verification of Eq.(18) or (22) requires that the forces that stabilize the dsDNA structure and have been included in the Hamiltonian (see Sec. II) be temperature independent and the separated strands of DNA do not form hair-pin or globule like structures.\[28\].

\[28\]
V. CONSTANT EXTENSION ENSEMBLE

A. Extension of one of the ends base pair

One can devise experiments in which the separation of one end of the two strands of dsDNA is kept fixed and the average force needed to keep this separation can be measured. In fact the experimental set up of Essevaz-Roulet [7] to unzip DNA by displacing the bead at constant velocity belongs to the category of the constant extension ensemble.

To calculate the force needed to keep one of the ends base pair of the chain to a given separation let us consider a chain of \( N \) base pairs of which the base pair 1 is stretched to a distance \( y \) and the \( N^{th} \) base pair is fixed to zero separation (i.e. \( y_N = 0 \)). The configurational partition function (see Eq. 6) of this chain is

\[
Z_N^c(y) = \left( \frac{\beta k}{2\pi} \right)^{N/2} \int \prod_{n=1}^{N-1} dy_n \delta(y_1 - y) \delta(y_N - 0) \times \exp \left\{ -\beta \sum_{n=1}^{N-1} \left[ \frac{1}{2}(V(y_n) + V(y_{n+1})) + w(y_n, y_{n+1}) \right] \right\} \tag{23}
\]

In terms of the eigenvalues and eigenfunction of transfer integral operator defined in Eq. (9) we can write Eq. (23) as

\[
Z_N^c(y) = e^{-\beta V(y)/2} \phi_0(y) e^{-\beta(N-1)\epsilon_0} \phi_0(y_N = 0) e^{-\beta V(y_N = 0)/2} \]

\[
= Ce^{-\beta V(y)/2} \phi_0(y) Z_N^c \tag{24}
\]

where the constant \( C = e^{\beta(\epsilon_0 - D)/2} \phi_0(y_N = 0) \) and \( Z_N^c = e^{-\beta N \epsilon_0} \).

The work done in stretching the first base pair to distance \( y \) is therefore (as the constant \( C \) is \( y \) independent, we drop it henceforth)

\[
W(y) = \frac{1}{2} V(y) - k_B T \ln \phi_0(y) \tag{25a}
\]

\[
= \frac{1}{2} V(y) - k_B T \left[ \ln Z_N^c(y) - \ln Z_N^c \right] \tag{25b}
\]

The derivative of \( W(y) \) with respect to \( y \) gives the average force \( F(y) \) that is needed to keep the extension equal to \( y \). Thus,

\[
F(y) = \frac{\partial W(y)}{\partial y} \tag{26}
\]
In Eqs. (24) & (25) \( V(y)/2 \) appears as an end term. This is because in constructing the transfer integral that connects the base pair 1 with the base pair 2 only \( V(y)/2 \) has been taken into account and remaining \( V(y)/2 \) acts as an end term. When the periodic boundary condition is imposed as in the study of thermal denaturation, the term \( V(y)/2 \) of base pair 1 and the term \( V(y_N)/2 \) of base pair \( N \) get absorbed in the transfer integral that connects the base pair 1 with the base pair \( N \) and therefore no end term appears.

For a homogeneous chain one can use the eigenfunction \( \phi_0(y) \) found by solving Eq. (9) to calculate \( W(y) \) from Eq. (25a). Alternatively, one can use the method of matrix multiplications to calculate the partition functions \( Z_N^c(y) \) and \( Z_N^c \) and use Eq. (25b) to find the value of \( W(y) \). The method of matrix multiplication is useful as it can be applied to cases (e.g. heterogeneous chains) where the method of transfer integral is not applicable.

In the method of matrix multiplication one first constructs a matrix for each base pair by using the potential parameters corresponding to the base pair under consideration. The discretization of the coordinate variables and introduction of a proper cutoff on the maximum value of \( y \)'s determine the size of the matrices and the number of base pairs in the chain the number of matrices to be multiplied [21, 22]. As in evaluation of eigenvalues and eigenfunctions from Eq.(9) we chose -5.0 and 195.0 Å as the lower and upper limits of integration and the Gauss-Legendre method to discretize the integral. We found that the number of grid point \( M = 450 \) leading to \( 450 \times 450 \) matrix for each base pair gives good result. We have considered chains of number of base pairs varying from 100 to 300 and compared their results in Table 1 and 2 at \( T = 200 \) K and 300 K, respectively for the potential parameters of set (ii). In Fig. 5 we compare the result found for \( W(y) \) and \( F(y) \) for a chain of 100 base pairs with that of the result found from Eq.(25a) using the eigenfunctions \( \phi_0(y) \). In view of excellent agreement found between the values calculated from the two methods we conclude that even a chain of 100 base pairs is good enough to calculate \( W(y) \) and \( F(y) \) and some other related properties (as discussed below) where the method of transfer integral is not valid. In such calculations one has, however, to be careful to keep the value of \( y \) sufficiently small compared to length of the molecule.

The force \( F(y) \) calculated using Eq. (26) is shown in Fig. 6 for both sets of potential parameters at \( T = 300 \) K. We note that the existence of a very large force barrier at short distance \( (y \sim 0.2 \text{ Å}) \) and large difference in the values of the force at small extension from the two sets of potential parameters. For example, the value of the force at the peak for the
FIG. 5: Comparison of (a) work done and (b) force needed to keep end base pair at a distance $y$ calculated using groundstate eigenfunction and matrix multiplication methods (Eqs. 25a & 25b). This curve is at $T = 300$ K.

The set of parameters (i) is about thrice that found for the set of parameters (ii). The width of the peak for the set of parameters (i) is also about thrice that of the set of parameters (ii).

TABLE I: Values of work $W(y)$ done in stretching one of the ends base pair of dsDNA molecule of $N$ base pairs to a distance $y$ at $T = 200$ K for potential parameters of set (ii).

| Work done (eV)          |  $N=100$ | $y=0.5$ Å | $y=1.0$ Å | $y=5.0$ Å | $y=10.0$ Å |
|-------------------------|---------|-----------|-----------|-----------|-----------|
| $y=0$                   | 0.0154  | 0.0397    | 0.2470    | 0.4296    | 0.4296    |
| $y=5$                   | 0.0154  | 0.0397    | 0.2470    | 0.4296    | 0.4296    |

As suggested in ref. [19], the physical origin of the large force barrier is in the potential well due to hydrogen bonding plus the additional barrier associated with the reduction in
TABLE II: Values of work $W(y)$ done in stretching one of the ends base pair of dsDNA molecule of $N$ base pairs to a distance $y$ at $T = 300$ K for potential parameters of set (ii).

| $N$ | $y = 0.5$ Å | $y = 1.0$ Å | $y = 5.0$ Å | $y = 10.0$ Å |
|-----|-------------|-------------|-------------|-------------|
| 100 | 0.0136      | 0.0327      | 0.1769      | 0.2801      |
| 300 | 0.0136      | 0.0327      | 0.1769      | 0.2801      |

FIG. 6: The average force $F(y)$ in pN required to stretch one of the ends base pair to a distance $y$ at $T = 300$ K. The minimal separation $y = 0$ corresponds to the dsDNA equilibrium structure. For sufficiently large value of $y$ the $F(y)$ approaches to the value found from the constant force ensemble and shown in Fig. 4. The dashed line corresponds to potential parameters of set (i) and full line to set (ii).

DNA strand rigidity as one passes from dsDNA to ssDNA. The large difference in the value of the force on the peak for the two sets of potential parameters is primarily due to large difference in the barrier associated with the reduction in the DNA strand rigidity. In other words, the value of the force at the peak depends rather sensitively on the value of $\rho$ and the value of the potential depth $D$. Beyond $y \sim 1.0$ Å the contribution arising due to the potential $V(y)$ becomes almost negligible compared to the contribution arising due to the term involving $\phi_0(y)$.

The single and double stranded portions of the molecule are separated by a boundary region. In this boundary region which may be of three or four base pairs length [19] the
FIG. 7: The on-site potential $V(y)$ as a function of displacements. The dashed line represents the Morse potential (see Eq. (4)) and the full line the potential chosen to represent the interaction at the defect sites.

bases are unpaired but not free to fluctuate. The peak corresponds to the energy needed to create this boundary. To test validity of this argument we calculated $F(y)$ curve by replacing first few base pairs by defects. A defect on a DNA chain means mismatched base pair [21]. For example, if one strand of DNA has adenine on a site, the other strand has guanine or cytocine or adenine in place of thymine on the same site. In such a situation the pair will remain in open state at all temperatures as the two nucleotide cannot join each other through hydrogen bonds. We therefore replace the on-site Morse potential by a potential shown in Fig. 7 by full line. This potential has repulsive part as well as the flat part of the Morse potential but not the well that arises due to hydrogen bonding interactions. Due to defect on a site the stacking interactions with adjacent bases will also be affected. Since the formation of hydrogen bonds changes the electronic distribution on base pairs causing stronger stacking interactions with adjacent bases. Therefore, when base pairs without hydrogen bonds are involved the stacking interaction will be weaker compared to the case when both base pairs are intact. This fact has been taken into account in our calculation by reducing the anharmonic coefficient $\rho$ to its half value whenever one of the two base pairs involved in the stacking interactions is defective and zero when both are defective.
FIG. 8: (a) The work $W(y)$ in eV done at $T = 200$ K in stretching one of the ends base pair to a distance $y$ and the change in the value of $W(y)$ when some of the base pairs including the one being stretched are replaced by defect base pairs are shown. In (b) the force $F(y)$ in pN at $T = 200$ K required to stretch one of the ends base pair to a distance $y$ in presence of defect base pairs is shown. The peak position is found to shift to larger values of $y$ as the number of defect base pairs is increased. The results plotted in this figure are obtained using the potential parameters of set (ii).

For given number of defect base pairs we calculated $W(y)$ and $F(y)$ using Eq.(24) and (25). The results are shown in Fig. 8 for $T = 200$ K and in Fig. 9 for $T = 300$ K.

As expected, the work needed to create a separation $y$ of an end base pair decreases as the number of defect base pairs increases. When $y$ becomes larger than certain value which, in turn, depends on the number of defect base pairs (see Figs. 8 & 9) $W(y)$ becomes equal to that of a defectless dsDNA. It also seems that for small $y$ the value of $W(y)$ attains a lower limit that is independent of the number of defect base pairs. This behaviour can also be seen...
FIG. 9: The curves in (a) and (b) are same as in Fig. 8 but for $T = 300$ K.

in $F(y)$ curve; as for small $y$ the value of $F$ is (except with no defect) almost same for all cases and for large $y$ the value of force approaches to its asymptotic value. It is interesting to note that the peak in $F(y)$ curve shifts to larger values of $y$ as the number of defect base pairs increases and the height of the peak decreases. These features can be understood from the fact that the barriers that give rise the peak in $F(y)$ curve shift to the base pairs after the segment of the DNA that contains defect base pairs. Note that this segment of DNA with defect base pairs is in single stranded form and can have comparatively large thermal fluctuations. The energy associated with this fluctuation (entropic) contributes to reducing the barrier that is responsible for the peak in $F(y)$ curve. As the length of this defect segment increases the entropic contribution increases and therefore more reduction in the height of the peak in $F(y)$ curve.

Since the effect of temperature is to reduce the effective height of barriers responsible for the peak and to increase the entropic contributions, the peak height in $F(y)$ curve are smaller at $T = 300$ K compared to that at $T = 200$ K.
In Table 3 we list the value of force at the peak and the peak position for a number of defect base pairs calculated using the potential parameters of set (ii). We note that the position of the peaks at $T = 200$ K are at larger values of $y$ compared to the corresponding peaks at $T = 300$ K. For example, for the first five base pairs being defective, the peaks are found for $y = 12.4\text{Å}$ and $8.8\text{Å}$, respectively at $T = 200$ K and $300$ K. This means, one has to have larger extension to encounter the force peak in $F(y)$ curves at lower temperatures compared to that found at higher temperatures. This can be understood from the fact that (as shown below) the extension at one end in a dsDNA creates a fork of Y shape and the length of this fork increases as extension $y$ increases. As we will see that to have same length of the fork one has to have larger extension at low temperature compared to that at high temperatures. Therefore to reach to those base pairs which are responsible for the barriers leading to peak in $F(y)$ one has to have relatively larger extension as temperature is lowered.

TABLE III: Peak position and the value of force at the peak for different number of defect base pairs located at one end of dsDNA.

| $N_d$ | T = 200 K | T = 300 K |
|---|---|---|
| | P. Position (in Å) | P. Height (in pN) | P. Position (in Å) | P. Height (in pN) |
| 0 | 0.19 | 235.77 | 0.19 | 226.48 |
| 1 | 3.6 | 96.33 | 3.2 | 70.57 |
| 3 | 8.4 | 89.53 | 6.4 | 59.90 |
| 5 | 12.4 | 84.60 | 8.8 | 55.04 |
| 11 | 22.8 | 77.22 | 14.0 | 49.11 |

To see the formation of a fork of Y shape on stretching the two strands of a dsDNA from one end we calculate $\langle y_n \rangle$ for $n > 1$ for the value of $y_1 = y$ from the relation

$$\langle y_n \rangle = \frac{\int \left( \prod_{i=1}^{N} dy_i \right) y_n \exp[-\beta \sum_{i=1}^{N} H(y_i, y_{i+1})] \delta(y_1 - y)}{\int \left( \prod_{i=1}^{N} dy_i \right) \exp[-\beta \sum_{i=1}^{N} H(y_i, y_{i+1})]} \tag{27}$$

We use matrix multiplication method to find $\langle y_n \rangle$ for a dsDNA of 200 base pairs. We have checked the accuracy of our results given below by using dsDNA of longer sizes and found that as long as $y$ is kept sufficiently small compared to the size of the chain, results remain
FIG. 10: The shape and size of fork of Y shape formed at $T = 200$ K (a) and $T = 300$ K (b) when one of the ends base pair is stretched. $\langle y \rangle$ measures the separation in angstrom of the two strands at different sites (base pairs) numbered from 1 to $N$.

independent of the size of the chain. In Fig. 10 we plot the values of $\langle y_n \rangle$ showing the average position of the two strands at different values of extension. The formation of Y fork at the end being stretched is clearly seen. The length of the fork increases on increasing the value of $y$. We also note that the effect of temperature on the length of the fork. For example, at $y = 5 \text{Å}$ the junction of the two branches of the fork is located at base pairs $n = 4$ and 6 respectively at $T = 200$ and 300 K. We consider $n^{th}$ base pair open if $\langle y_n \rangle$ is equal or greater than 1Å and bound or intact if value of $\langle y_n \rangle$ is less than 1Å. In Fig. 11 we plot the number of open base pairs as a function of extension $y$. We note that except for very small values of $y$ the number of open base pairs increases linearly with the extension and the slope of the line corresponding to 300 K is about twice as compared to that for 200 K.
FIG. 11: The number of open base pairs, \( N_o \), as a function of the extension of the end base pair at temperature \( T = 200 \text{ K} \) (full line) and 300 K (dashed line). A base pair is considered to be open when the value of \( \langle y \rangle \) is equal or greater than 1 Å and closed if it is less than 1 Å. At initiation of unzipping the number of base pairs that get open depends on the anharmonic term in the stacking interaction (see Eq. 5) but after some extension it depends linearly on \( y \). The slope of the curve at \( T = 300 \text{ K} \) is about twice the corresponding value at \( T = 200 \text{ K} \).

B. Extension of a base pair in the middle

In replication the opening of dsDNA is initiated at one of the ends whereas in case of the transcription it can be anywhere. It is therefore of interest to investigate the formation of bubble of ssDNA in a dsDNA away from the ends.

We consider the situation in which a bubble of ssDNA is formed in the middle of a dsDNA by stretching a base pair and calculate the average force needed to create it. For this we use the method of matrix multiplication described above and calculate the work \( W(y_m) \) done in stretching the middle base pair by a distance \( y_m \) from the relation (see Eq.(24))

\[
W(y_m) = -k_B T (\ln Z_N^c(y_m) - \ln Z_N^c)
\]  

(28)

where \( Z_N^c(y_m) \) is the partition function of dsDNA of \( N \) base pairs with middle base pair kept at \( y_m \) separation. For our calculation we have taken \( N = 200 \) as we pointed out earlier that as long as \( y_m \) is small the results are independent of length of the molecule and constructed the matrix using the procedure already described.
FIG. 12: Comparison of (a) the work $W(y)$ that has to be done and (b) the average force $F(y)$ needed when a base pair of one of the ends or in the middle of the molecule is stretched to a distance $y$. The results plotted here correspond to potential parameter of set (ii) at $T = 300 \text{ K}$.

The values of $W(y)$ and $F(y)$ found from this calculation are plotted in Fig. 12 and as shown in the figures the values for any given extension are exactly twice the values when one of the ends base pair was stretched only when $y \geq 1.0 \text{ Å}$ but not for $y < 1.0 \text{ Å}$. This is because for the extension $y < 1.0 \text{ Å}$ a contribution due to $V(y)/2$ arises in the case of extension of one of the ends base pair but not in the case of extension of a base pair away from the ends. The reason why one gets for $y \geq 1.0 \text{ Å}$ the force needed to stretch a base pair in the middle twice that of the base pair at one ends is due to the fact that a bubble of open base pairs formed in the middle has to propagate on both sides in contrast to the earlier situation in which it has to move in one direction only.

Alternatively, we can use the argument used in writing Eq.(25a). As the probability of finding the middle (or for that matter any base pair away from the ends) at a separation of
FIG. 13: The curves in (a) and (b) are same as in Fig. 9 with a difference that instead of a base pair at the end, a middle base pair of a dsDNA molecule is stretched and defect base pairs are introduced symmetrically about the middle base pair. The results plotted here are for $T = 300$ K and correspond to the potential parameter of set (ii).

$y$ is $|\phi_0(y)|^2$, the work done in achieving the extension $y$ is, therefore,

$$W(y) = -2k_B T \ln \phi_0(y)$$  \hspace{1cm} (29)

Note that this term is twice the second term in Eq. (25a) only. Since the contribution arising due to first term in Eq. (25a) is only for small values of $y$ (i.e. $y < 1.0$ Å), the work done in pulling a base pair that is far away from the ends, is twice the work done in pulling one of the ends base pair for $y \geq 1.0$ Å. The asymptotic value of the force which is equal to the one found from the constant force ensemble is, however, exactly two times to the value corresponding to the extension done at the one end.

The effect of defects on $W(y)$ and $F(y)$ has been calculated by making the base pair that is being stretched and others symmetrically on both sides of it as defect base pairs. The
result found for different number of defect base pairs are shown in Fig. 13 for $T = 300$ K. Comparing the results of this figure with those of Fig. 9 we find that the qualitative nature of $W(y)$ and $F(y)$ curves in these two cases is similar except in the case when one base pair (i.e. the base pair which is being stretched) is a defect base pair. It can be seen that the peak position in $F(y)$ in Fig. 9 (and Table 3) is shifted from 0.19 Å to 3.2 Å whereas in the case shown in Fig. 13 there is very little shift. This means that in the latter case the position of the barrier that gives rise the peak in $F(y)$ does not shift. The decrease in the value of force at the peak is primarily due to loss of energy of hydrogen bonds in the base pair. The other important point to be noted is that in the presence of defects the peaks in $F(y)$ is more than twice the corresponding peak in Fig. 9. This can be understood from the fact noted above that decrease in the height of peak is due to contribution arising from thermal fluctuations in the segment of DNA containing defects. This contribution is larger when the segment is located at the end of the chains than in the middle. In Table 4, we list the position and height of the peak in $F(y)$ curve of these two cases for $T = 300$ K. We note that the peaks occur at smaller values of extension compared to the case of end extension.

TABLE IV: Comparison of the peak position and the values of force at the peak for different number of defect base pairs introduced at one end and in the middle of dsDNA at $T = 300$ K.

| $N_d$ | Defect in middle | Defect at end |
|------|-----------------|---------------|
|      | P. Position (in Å) | P. Height (in pN) | P. Position (in Å) | P. Height (in pN) |
| 0    | 0.19            | 239.43        | 0.19            | 226.48        |
| 1    | 1.25            | 148.77        | 3.2             | 70.57         |
| 3    | 5.1             | 127.67        | 6.4             | 59.90         |
| 5    | 6.3             | 120.95        | 8.8             | 55.04         |
| 11   | 8.7             | 107.52        | 14.0            | 49.11         |
| 15   | 12.0            | 102.33        | 19.2            | 46.13         |

The value of $\langle y_n \rangle$ calculated from Eq.(26) with the modification that now the middle base pair is kept at fixed value are plotted in Fig. 14. Formation of an “eye shape” bubble in the middle is clearly seen. The length of the bubble increases symmetrically as the extension of the middle base pair is increased.
FIG. 14: Formation and elongation of a bubble of ssDNA with a shape of an eye at $T = 300$ K when a base pair far away from the ends is stretched. The results plotted corresponds to potential parameters of set (ii) at $T = 300$ K.

VI. SUMMARY AND CONCLUSION

The basic features of unzipping of a dsDNA molecule under the influence of external force have been investigated using the PB model. The model, though ignores the helicoidal structure of dsDNA molecule, has enough details to analyze the mechanical response at the few Å scale and is simple enough for numerical and analytical analysis.

The critical force $F_c(T)$ for unzipping calculated in the constant force ensemble is found to depend on the potential parameters $k$ and $D$ measuring, respectively, the stiffness of a single strand of DNA and the depth of the on-site potential. The temperature dependence of $F_c(T)$ is found to be $(T_D - T)^{1/2}$ where $T_D$ is the thermal denaturation temperature in absence of the external force. It is, however, important to note that our approach assumes that the interactions that stabilize the dsDNA structure and are included in the PB model are temperature independent and separated strands of DNA do not fold to form hair-pin or globule-like structures. Both these assumptions may not strictly be met in systems commonly used in experiments [28]. Therefore, experimental verification of Eq.(22) needs special care.

In the constant extension ensemble the average force $F(y)$ needed to stretch a base pair $y$ distance apart is found to have a large barrier at the separation of the order of 0.2Å. A similar result has been reported by Cocco et al [19]. The value of $F(y)$ for any $y$ depends
on whether the base pair being stretched is one of the ends base pair or located far away from the ends of the molecule. The value of $F(y)$ of the latter case for a given extension $y$ is found exactly twice of the value of the former case for the same extension except for extension less than $\sim 1.0 \text{ Å}$ (Fig. 12).

When the two strands of a base pair are stretched to some distance apart it forces a part of the molecule into single stranded form and therefore creates a boundary region separating the single and double stranded positions. The base pairs of boundary region are open in the sense that the hydrogen bonds are broken but the strands are not free to fluctuate because of neighbouring bound base pairs. The peak in $F(y)$ corresponds to the energy needed to create this boundary and its value is shown to depend on $k\rho$ and $D$. The quantity $k\rho$ measures the barrier associated with the reduction in DNA strand rigidity as one passes from dsDNA to ssDNA. The value of $F(y)$ at the peak for the potential parameters of set (i) is about three times larger than that found for the set (ii); the reason being the large difference in the value of $k\rho$ of the two sets of potential parameters.

The measurements of the value of force at the peak in the $F(y)$ curve of a dsDNA molecule is, however, difficult as most experiments are carried out under conditions where stretching of a base pair cannot be controlled on the Å scale. However, as we have shown in Figs. 8, 9 and 13, the peak in the $F(y)$ curve can be made to occur at larger values of the extension $y$ by replacing some of the base pairs by defect base pairs. From these results it therefore seems possible to use defects to create the force barrier at such extensions of a base pair that the peak in $F(y)$ curve can be measured directly in an experiment.

The on-site potential on a defect site is represented by a potential that has only a short-range repulsion and a flat part without well of the Morse potential (Fig. 7). The coefficient $\rho$ of the anharmonic term of the stacking interaction containing defect base pairs is also suitably modified. With these modifications in the PB model and with the potential parameters of set (ii) we have calculated the $F(y)$ curves for different number of defect base pairs. The results shown in Figs. 8 & 9 are for the case in which one of the ends base pair was stretched and in Fig. 13 for the case when the middle base pair was stretched. The qualitative features of the curve $F(y)$ in the two cases are similar except in the case when one base pair (i.e. the base pair which is being stretched) is a defect base pair. This is because the barrier that gives peak in $F(y)$ curve in this case remains at the same location as in the defectless case; the decrease in the value of force at the peak is primarily due to
loss of hydrogen bonds energy in the base pair.

It has been found (see Table 4) that in the presence of defects the peaks in $F(y)$ shown in Fig. 13 is more than twice the corresponding peak in Fig. 9. This has been attributed to difference in the entropic contributions which reduces the barrier height that gives the peak in $F(y)$ curve.

Stretching a base pair at one of the ends of a dsDNA molecule creates a fork of Y shape which moves along the chain on increasing the extension, $y$, of the end base pair. Its size for a given extension $y$ depends on the temperature as shown in Fig. 14. The number of base pairs that get open on initiation of the formation of the fork depend rather sensitively on the anharmonic term in the stacking energy. However, after certain size of the fork the number of base pairs that get open depend linearly on the extension $y$ as shown in Fig. 11.

When the middle (or any base pair far away from the ends) base pair is stretched a bubble of ssDNA with the shape of an eye is formed. In a homogeneous dsDNA molecule the bubble move symmetrically on both sides on increasing the extension of the middle base pair. This may not, however, happen in case of a heterogeneous dsDNA molecule. The method of matrix multiplications used in calculating the properties in the constant extension ensemble can also be applied to a heterogeneous dsDNA molecule.

Acknowledgement

The financial support from Council for Scientific and Industrial Research (CSIR) and Department of Science and Technology (DST), Government of India, New Delhi is acknowledged.

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