Unzipping DNA - towards the first step of replication

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DNA, the basic genetic material, is generally a very long, flexible, linear or circular molecule, with length varying from 2μm (5000 base pairs) for simple viruses to 3.5 × 10^7μm (∼ 10^{11} base pairs) for more complex organisms. In spite of the wide diversity in the information content and the functionalities of organisms, the general rules for replication have an astounding universality in the sense of independence of the system. A double helical DNA can be made to melt (i.e. the two strands can be separated) in vitro (i.e. in the lab) by changes in pH, solvent conditions and/or temperature (“thermal melting”), but they are found to be extremely stable in the cell. As per the standard dogma of molecular biology, every step of a biochemical process is mediated by an enzyme (encoded in the DNA), and the process “that accompanies DNA replication requires enzymes specialized for this function: DNA helicases to disrupt the base pairs, topoisomerases to provide a swivel to unwind the strands, and single-strand binding proteins (SSB’s) to coat and stabilize the unwound strands. Melting of the duplex at a replication origin for initiation, and at the fork during elongation, requires an expenditure of energy, an investment justified by the functional and evolutionary benefits of maintaining the genome as a duplex DNA.”

Many biochemical details of the replication process are known, organism by organism. The proposal of a Y-shaped structure \[\text{(a)}\] for a linear molecule \[\text{(b)}\] as the starting point of replication seems to be corroborated by experiments \[\text{[3]}\], but as yet a proper analytical understanding is lacking. Very recently, with the advent of various physical techniques, attempts have been made to study the process from a physical, rather than a biochemical, point of view. Special attention has been given to the measurements of forces to unzip a double stranded DNA (dsDNA) molecule. Several studies have been made to understand the effect of forces in absence of any enzymes \[\text{[4–7]}\], while, in other studies, enzymatic activities involving expenditure of energy and displacement have been interpreted in terms of effective forces \[\text{[8]}\]. Similar to “transcription against applied force” of Ref. \[\text{[9]}\], experiments combining the mechanical opening of DNA with the enzymatic replication or transcription have been proposed \[\text{[10–12]}\], and such an experiment would be highly significant. The results of Ref. \[\text{[10]}\] distinguish the cases of conventional thermal melting and the unzipping which has been called directional melting \[\text{[8]}\]. Our analytical approach shows the differences between these two cases.

Some of the quantitative questions are the following:

1. Is there a critical force to open up a double-stranded chain in thermal equilibrium, acting say at one end only?
2. Is the nature of the transition different from the thermal melting of the bound pair and is it reflected in the opened region? Once these equilibrium questions are understood, we may ask 3) how the pair opens up in time after the force is applied - the question of dynamics of unzipping.

In order to focus on the effect of the pulling force (see Fig. 1) on the bound strands, we take the viewpoint of a minimal model that transcends microscopic details but on which further details can be added for a realistic situation. Our approach differs from the previous studies \[\text{[13–17]}\] in the emphasis on the opening of the fork. We treat the DNA as consisting of two flexible interacting elastic strings. Winding is ignored. In a sense, the effect of topoisomerases is built into the model by maintaining the chains in the native state. Such a model has been found to be useful for many properties of DNA \[\text{[13–17]}\]. For quantitative results we consider a bound situation as obtained by a binding square-well potential. Our focus is on the simpler aspects of the theory, and here we answer the first two questions posed above.
Our model is this: Two gaussian polymer chains \([18]\) in \(d = 3\) dimensions \([19]\), with \(\tau\) denoting the position of a monomer along the contour of a chain, are tied at one end and pulled by a stretching force \(\mathbf{g} = g \hat{e}_g\) at the other end (see Fig. 1), \(\hat{e}_g\) being the unit vector in the direction of the force. The energy from the force is proportional to the separation \(r(N)\) of the end points at \(\tau = N\), i.e.

\[
-g \cdot r(N) = - \int_0^N d\tau g \cdot \frac{\partial r(\tau)}{\partial \tau},
\]

since \(r(0) = 0\). Assuming identical interaction for all base pairs (or for a DNA with identical base pairs) the Hamiltonian can be written in the relative coordinate \([20]\)

\[
\frac{H}{k_BT} = \int_0^N d\tau \left[ \frac{1}{2} \left( \frac{\partial r(\tau)}{\partial \tau} \right)^2 - g \cdot \frac{\partial r(\tau)}{\partial \tau} + V(r(\tau)) \right],
\]

where \(r(\tau)\) is the \(d\)-dimensional relative coordinate of the two chains at the contour length \(\tau\). Base pairings require that the monomers on the two strands interact only if they are at the same contour length \(\tau\). The potential energy is therefore given by the integral of the potential \(V(r(\tau))\) over \(\tau\). \(V(r)\) is a short-range potential and its detailed form is not important. One choice, as generally used for renormalization group approach, is to take a contact potential \(V(r) = \psi_0 \delta_m(r)\) where, in Fourier space, \(\delta_m(q) = 1\) for \(|q| \leq \Lambda\), \(\Lambda\) being a cut-off reminiscent of the underlying microscopic structure. For the equivalent quantum mechanical calculation, we choose a square-well potential. In thermal equilibrium, the properties are obtained from the free energy \(F = -k_BT \ln Z\) where the partition function \(Z = \int DR \exp(-H/k_BT)\) sums over all the configurations of the chains.

The Hamiltonian written in the above form can be thought of as a directed polymer in \(d + 1\) dimensions about which many results are known \([21,22]\) for \(g = 0\). If we treat \(\tau\) as a time like co-ordinate, then the same Hamiltonian represents, in the path integral formulation, a quantum particle in imaginary time. This quantum particle with \(g \neq 0\) then corresponds to the imaginary vector potential problem discussed in recent times \([24]\). We make use of both the pictures in this paper and follow the formulation of Ref. \([24]\) closely.

For \(d = 1\), the quantum problem with \(V(x) = -\psi_0 \delta(x)\), is exactly solvable, and is done in Ref. \([23]\) as a single impurity problem. It was shown that there is a critical \(g_c\) below which the force does not affect the bound-state energy, i.e. the quantum particle remains localized near the potential well, while for \(g > g_c\) the particle delocalizes. In the polymer picture, this means that in low dimensions, a force beyond a critical strength separates the two strands. It should be pointed out here that the force is applied at one end only, and but it is not a boundary or edge effect mainly because of the connectivity of the polymer chain as expressed by Eq. 1.

Details of the phase transition behavior of the Hamiltonian of Eq. 3 for \(g = 0\) are known from exact renormalization group (RG) calculations \([21,22]\) for a delta-function potential. With \(g = 0\), there are two fixed points (fp) at (i) \(u^* = 0\), and (ii) \(u^* = \epsilon\), with \(\epsilon = 2 - d\) and \(u = vL\) as the dimensionless running potential strength parameter, \(L\) being an arbitrary length-scale. For \(d < 2\), the first one is unstable and hence the critical point while for \(d > 2(\epsilon < 0)\), the second one is the unstable one. The unstable fp \((u_n^*)\) represents the melting or unbinding of the two chains. It also follows \([23]\) that other details \([24]\) of \(V(r)\) are irrelevant in the RG sense, i.e., they vanish in the large length-scale limit, explaining the universality of the problem.

The important length-scales for this critical point, from the bound state side, comes from the typical size of the denatured bubbles of length \(\tau_m\) along the chain and \(\xi_m\) in the spatial extent (Fig. 1b). Close to the critical point these length scales diverge as \(\xi_m \sim \Delta u \sim \nu_m\) and \(\tau_m \sim \xi_m^\zeta\), where \(\Delta u\) is the deviation from the critical point, where \(\nu_m\) and \(\zeta\) are the two important exponents \([21]\).

\[
\nu_m = 1/|d - 2| \quad \text{and} \quad \zeta = 2.
\]

It is this \(\zeta\) (the dynamic exponent of the quantum problem) that will distinguish the new phenomena we are trying to understand \([25]\).

The mapping to the imaginary time quantum mechanics allows us to use the methods of quantum mechanics \([23]\). As mentioned, we choose a square-well potential \(V(r) = -V_0\), for \(r < r_0\), and 0, otherwise. The quantum Hamiltonian is then given by \([23]\)

\[
H_q(g) = \frac{1}{2} (\mathbf{p} + ig)^2 + V(r),
\]

in units of \(\hbar = 1\) and mass \(m = 1\), with \(\mathbf{p}\) as momentum. For simplicity, the well is chosen to be just deep enough to have only one bound state (in the quantum mechanical picture, with \(g = 0\)) with energy \(E_0 < 0\). The non-hermitian Hamiltonian can be connected to the hermitian Hamiltonian at \(g = 0\) by

\[
U^{-1} H_q(g) U = H_q(g = 0), \quad \text{where} \quad U = \exp(g \cdot r).
\]

The wave-functions are also related by this \(U\)-transformation so that if the transformed bound (i.e. localized) state wave-function remains normalizable, the bound state energy will not change. We refer to Ref. \([23]\) for details of the argument. The continuum part of the spectrum will have the minimum energy \(E = -g^2/2\) (the state with wave-vector \(k = 0\)). For the localized state at \(g = 0\), the wave-function for \(r > r_0\) is \(\psi_0(r) \sim \exp(-kr)\) where \(k = 2\sqrt{|E_0|}\). The right eigenvector for \(H_q(g)\) is then \(\psi_0(r) \sim \exp(g \cdot r - kr)\), obtained via the \(U\)-transformation. This remains normalizable if \(g < k\), so
that the binding energy remains the same as the \( g = 0 \) value until \( g = g_c \equiv \kappa \). The generic form of the spectrum is shown in Fig. 2a. This indicates a delocalization transition by tuning \( g \) - the unzipping or the directional melting of DNA at \( g = g_c \). The phase diagram is shown schematically in Fig. 2b. There is a gap in the spectrum (Fig. 2a) for \( g < g_c \) and the gap vanishes continuously as \( |g^2 - \kappa^2| \sim |g - g_c| \) as \( g \to g_c^- \). Since the time in the quantum version corresponds to the chain length, the characteristic chain length for the delocalization transition is therefore \[ \tau_{\text{dm}}(g) \sim |g_c - g|^{-1} \] (6)

The spatial length-scale of the localized state is determined by the width of the wave-function and, for \( g = 0 \), it is set by \( \kappa^{-1} \). For \( g \neq 0 \), the right wave-function, \( \psi_R \), has a different length scale and this length-scale diverges as the wave-function becomes non-normalizable. The width of \( \psi_R \) gives this scale as

\[ \xi_{\text{dm}}(g) \sim |g_c - g|^{-\nu_{\text{dm}}} \quad \text{with} \quad \nu_{\text{dm}} = 1, \] (7)

for \( g \to g_c^- \). We find \( \tau_{\text{dm}} \sim \xi_{\text{dm}} \) and therefore

\[ \zeta = 1. \] (8)

![FIG. 2. (a)The energy spectrum for \( g \neq 0 \) for the Hamiltonian in Eq. (3). (b) The phase diagram in the \( u - g \) plane. Thermal melting takes place along the \( g = 0 \) line at \( u = u^*_u \). The hatched line indicates the unzipping transition or directional melting line. The biochemical reactions are conjectured to occur at a slight subcritical region indicated by the hatched region.](image)

The significance of \( \tau_{\text{dm}} \) can be understood if we study the separation of the two chains, i.e., \( \langle r \rangle_{\tau} \), at a distance \( \tau \) along the chain below the pulled end. This can be evaluated by using the standard rules of quantum mechanics. For infinitely long chains in the sub-critical region, only the bound and the first excited states are sufficient for the computation. One finds, along the pulled direction,

\[ \langle r \rangle_{\tau} \sim \exp[-\tau/\tau_{\text{dm}}(g)], \] (9)

where \( \tau_{\text{dm}}(g) \) is given by Eq. (6). In other words, \( \tau_{\text{dm}}(g) \) and \( \xi_{\text{dm}}(g) \) describe the unzipped part of the two chains near the pulled end (Fig. 1c). These length scales diverge with \( \zeta = 1 \) as the critical force is reached from below. (One can also define similar length-scales for \( g \to g_c^+ \) where the lengths would describe the bound regions.) The exponential fall-off, Eq. (3), of the separation from the pulled end immediately gives the picture of the replication Y-fork as shown in Fig. 1c.

Let us study the behavior of the free energy. Since the partition function for the Hamiltonian of Eq. (3) obeys a diffusion-like equation \[ \frac{\partial F}{\partial \tau} = 1 \left\{ 12 \right. \frac{\partial^2 F}{\partial \tau^2} - \frac{1}{2} (\nabla F)^2 - g \cdot \nabla F - v_0 \delta_{\Lambda}(r) \right\}, \] (10)

The left hand side represents the free energy per unit length of the chains. Under a scale transformation \( x \to bx \), and \( \tau \to b^c \tau \), the total free energy remains invariant so that the above equation takes the form

\[ \frac{\partial F}{\partial \tau} = 1 \left\{ 12 \right. \frac{\partial^2 F}{\partial \tau^2} - \frac{1}{2} (\nabla F)^2 - b^{c-2} g \cdot \nabla F - b^{c-3} v_0 \delta_{\Lambda}(r) \right\}. \] (11)

For \( g = 0 \), Eq. (11) tells us that \( \zeta = 2 \) and \( d = 2 \) are special for melting transition as we see in Eq. (3). For the choice \( \zeta = 1 \), the \( g \)-dependent term dominates and all other terms become irrelevant for large length-scale \( b \). It is this feature that shows up in the Y-fork of the unzipped chain. The robustness of Eqs. (11) also follows from this. With the dependence on the potential strength entering only through \( g_c \), these are valid along the hatched line of Fig. 2b, and could be oblivious to the details of the nature of the melting transition. In our simple model, the melting point appears as a multi-critical point in the phase diagram of Fig. 2b.

Now we see the striking difference between the directional melting and thermal melting (Fig. 1). In the thermal case the bubbles will have anisotropic shape with the spatial extent scaling as the square-root of the length along the chain. This is the characteristic of the fixed point \( u^*_c \) while the pulled case is described by a different scaling. The exponential profile of the Y-fork and its scaling are the two important characteristic features which should be experimentally verifiable.

We have considered only the equilibrium situation. The dynamics of this process of directional melting or unzipping is important. Similarly the condition of identical interaction may not be realistic for real DNA. This feature can be cured by taking the interaction \( v_0 \) to be random with a specific distribution. Such a random case also shows a different type of melting behavior. The mapping to a quantum problem is also then lost. Self-avoidance and other topological constraints can also be added to this model, though at the cost of the simplicity of the model. For example, self-avoidance can be introduced in Eq. (2) by adding a term...
\[ \frac{1}{2} \int d\tau \int d\tau' \nu_s \delta(\mathbf{r}(\tau) - \mathbf{r}(\tau')) \], with \( \nu_s > 0 \). The generalization to a two-chain problem is straightforward. Such a term can be “unsquared” by introducing an annealed gaussian-random potential \( V(r) \) with zero mean and variance \( \langle V(r)V(r') \rangle = \nu_s \delta(\mathbf{r} - \mathbf{r'}) \) so that the equivalent quantum hamiltonian is \( H_q = H_q + iV(r) \), where \( H_q \) is given by Eq. \[ \text{4} \]. This involves both an imaginary vector potential and an imaginary, random, scalar potential. The polymer problem is recovered from the \( V \)-averaged propagator of the quantum particle \[ \text{23} \]. Such a general non-hermitian hamiltonian is little understood at present even for the pure case, let alone the random one. We wish to come back to these issues in future.

Let us summarize our results and the emerging picture. There is a critical strength of the force required to unzip a double stranded DNA, and this directional melting is a critical phenomenon. Thermal or other fluctuations can open up regions along the length of the chain (bubbles with \( \zeta = 2 \)) but the unzipped part is characterized by a different scaling (\( \zeta = 1 \)), as shown in Fig. 1. In other words, the Y-fork created by the force represents a correlated region which is easily distinguishable from a thermal bubble. Living organisms probably work at a slightly sub-critical regime with \( g < g_c \) to take advantage of and exploit the distinct correlations for the enzymatic actions within the Y-fork. This can be achieved by coupling the biochemical reactions (like polymerization, unwinding etc) to the unzipping phenomenon, analogous, in spirit, to reaction-diffusion systems. The near-critical features of unzipping or directional melting then can lead to a coherent phenomenon we see as replication - a process requiring highly cooperative functioning of different enzymes in space and time. This way of viewing the correlated biochemical events has not been studied so far.

As a first step, we therefore suggest that high precision measurements be done to get the profile along the unzipped region of DNA (or simpler double-stranded polymers) under a pulling force, in vitro or in vivo.

I thank ICTP for warm hospitality, where a major part of this work was done. Acknowledgments will probably belittle the influence of a discussion with Mathula Thangarajh that shaped the final form of this work.

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\[ \frac{dL}{d\tau} = cu - u^2 + c_1 g^2 \], and \[ \frac{dL}{d\tau} = g(1 - c_2 u) \],

where \( g = gL \) is the dimensionless force and \( c_1, c_2 \) are numerical constants. These equations do reproduce the \( g = 0 \) flow equations and have flows that are consistent with the behavior obtained using the quantum mapping. This is under investigation.

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