Protein-mediated loops and phase transition in nonthermal denaturation of DNA

Karen G Petrosyan and Chin-Kun Hu
Institute of Physics, Academia Sinica, Nankang, Taipei 11529, Taiwan
E-mail: pkaren@phys.sinica.edu.tw and huck@phys.sinica.edu.tw

Received 26 October 2010
Accepted 10 December 2010
Published 6 January 2011

Online at stacks.iop.org/JSTAT/2011/P01005
doi:10.1088/1742-5468/2011/01/P01005

Abstract. We use a statistical mechanical model to study nonthermal denaturation of DNA in the presence of protein-mediated loops. We find that looping proteins which randomly link DNA bases located at a distance along the chain could cause a first-order phase transition. We estimate the denaturation transition time near the phase transition, which can be compared with experimental data. The model describes the formation of multiple loops via dynamical (fluctuational) linking between looping proteins, which is essential in many cellular biological processes.

Keywords: solvable lattice models, classical phase transitions (theory), mechanical properties (DNA, RNA, membranes, bio-polymers) (theory)
Denaturation of DNA is a fundamental biological process before the transcription stage [1]. Thermal denaturation of DNA [2] has been modeled in many ways, including the ladder [3], Poland–Scheraga [4] and Peyrard–Bishop [5] models. The process still attracts attention of theoreticians in an attempt to describe it most efficiently [6]. Besides melting, DNA also denatures under the influence of other factors such as pH value, salt concentration, other chemical factors, and mechanical forces. One example of the latter is the DNA denaturation induced by an externally applied torque. Experiments with single DNA molecules under torsional stress were reported in [7, 8] that shed more light on the mechanical properties of DNA molecules in connection with their functioning in living cells. \textit{In vivo} torque is exerted by the RNA polymerase that causes transcription-generated torsional stress [9] (see also [10] where a direct observation of DNA rotation during transcription by \textit{Escherichia coli} RNA polymerase was reported). A theoretical study of torque-induced DNA denaturation was presented by Cocco and Monasson [11] and a thorough investigation of the effect of mechanical forces and torques on DNA and its denaturation was made by Marko [12].

Here we are interested in nonthermal denaturation of DNA that precedes the transcription process. Transcription regulation typically involves the binding of proteins over long distances on multiple DNA sites which are then brought close to each other to form DNA loops [13]. The DNA loops can be formed by protein complexes, e.g., by the regulators of bacterial operons, such as ara, gal, and lac, and human proteins involved in cancer, such as retinoid X receptor. The presence of protein-mediated loops is also important for many other cellular processes, including DNA replication, recombination, and nucleosome positioning as was extensively discussed in [14].

Recently Vilar and Saiz [15] studied multiprotein DNA looping. They developed a model of formation of a single loop via connection of an arbitrary large number of proteins. Their model describes a switchlike transition between looped and unlooped phases, and has been extended to account for multiple loops [14]. Dynamic protein-mediated loops within the framework of molecular systems biology were considered in [13] for the cases of the lac operon and phage \textit{\lambda} induction switches. Here we consider a different model to describe the denaturation of DNA, which has loops formed by proteins that link bases randomly located along the molecular chain. Thus our model accounts for the formation of multiple loops that is essential in cellular biological processes like pre-mRNA splicing [1]. Yet another important feature of our model is that it presents a dynamic rather than static picture of formation of loops as the protein-mediated links between the base pairs fluctuate, i.e. the proteins couple and decouple in the course of time. This demonstrates a connection between formation of the structure of protein-mediated loops for the particular DNA–protein node-link interaction network and co-evolutionary complex networks [16, 17]. We show below that looping proteins can make the nonthermal denaturation process be a first-order phase transition. This is due to the effective long-range interactions by the mediating proteins. We are primarily interested in the phase transition, in the metastability phenomenon that we have found and in the kinetics of the denaturation. We then calculate the transition time from the double helix state to the coil state, which can be compared with experimental data.

\textit{The model.} Lattice models proved to be useful in studies of the phenomenology of DNA denaturation [18]. Here we consider a simple statistical mechanical model defined on a one-dimensional lattice with each site corresponding to a rung of the ladder [3]. A spin variable $\sigma_i$ is associated with each site $i$ where $\sigma_i = -1$ when the corresponding H-bond
is intact and \( \sigma_i = +1 \) when it is broken. We assume an arbitrary folding of the DNA molecule so that any two base pairs may get connected via the looping proteins. The proposed model has the following Hamiltonian:

\[
H = -g \sum_{i=1}^{N-1} \sigma_i \sigma_{i+1} - \gamma \sum_{i<j} J_{ij} p_i p_j \sigma_i \sigma_j - h \sum_{i=1}^N \sigma_i - \epsilon \sum_{i=1}^N p_i + \alpha \sum_{i<j} J_{ij}
\]

where \( g > 0 \) is the coupling parameter of nearest-neighbor interactions; \( J_{ij} \) are the link variables, taking values 0 and 1 when the \( i \) and \( j \) nodes are uncoupled or coupled by the proteins, correspondingly; the absence or presence of proteins at site \( i \) is defined by the variable \( p_i \) that takes values 0 or 1, respectively; \( \gamma > 0 \) is the energy of interaction between the base pair sites coupled via a link that has appeared caused by the on-site proteins; \( h \) is the binding energy between base pairs that includes the energy of the hydrogen bonds; \( \epsilon \) is the energy of binding of a protein at the site \( i \); \( \alpha \) is the energy of formation of a link connecting \( i \) and \( j \) sites; \( N \) is the number of base pairs.

The first term assures that broken pairs tend to break pairs next to them and in the same way it makes bases pair up next to paired ones. The second term describes the creation of links between proteins bound to bases at random sites of the molecule. These links form protein-mediated loops. The links actually fluctuate as the proteins at different sites may couple and decouple in the course of time. In general, \( \gamma \) may depend on the length of the loop [19]. However, such a dependence is a higher order effect and we do not consider that. In the third term the energy \( h \) depends on the external parameters that are determined by environmental conditions such as temperature \( T \), pH value, salt concentration, and other chemical as well as mechanical factors. Change in \( h \) may cause openings and closings of base pairs. As an example we will consider its dependence on an externally applied torque. The energy \( h \) is a sum of contributions from the base pairing energy \( h_0 < 0 \) and from the torsional energy \( h_r \), associated with a change in the local twist, that is \( h = h_0 + h_r \), where \( h_r = (1/2)C(\Delta \omega)^2 \), with \( C \) being the twisting elastic constant (torsional stiffness) and \( \Delta \omega = \omega - \omega_0 \) being the deviation of the spatial angular frequency \( \omega \) (change of the rung angle around the axis per unit length along the chain) from its unstressed value \( \omega_0 \) [20]. The torsional energy can also be represented via the torque \( \tau \) as \( h_r = \tau \varphi_0 \), where \( \varphi_0 = 2\pi/10.5 = 0.6 \) radians per base pair (a double helix contains about 10.5 base pairs per helical turn). The fourth term is the energy of binding of proteins. The last (fifth) term is the energy of formation of a link between base pairs mediated by the looping proteins. We will use another parameter \( c \) defined via \( c/(N - c) = e^{-\beta \Delta} \), where \( \beta \) is the inverse temperature. The ratio can be roughly treated as the probability of a link formation (see [16] for more rigorous formulations and details of a related model that describes a network of fluctuating links). We will assume sparse connectivity, \( c/N \ll 1 \), with the number of looping proteins much less than the number of bases. The Hamiltonian may also include long-range direct H-bond interactions between open base pairs via a term proportional to \( \sum_{ij} A_{ij}(1 + \sigma_i)(1 + \sigma_j) \) with an interaction matrix \( A_{ij} \). However we neglect these interactions assuming that their contribution is smaller compared to the interactions mediated by proteins\(^1\).

\(^1\) The energy of protein–protein interactions is estimated to be of the order of 10–20 kcal mol\(^{-1}\) [21] compared to the energy of a few kcal mol\(^{-1}\) for hydrogen bonds formed between DNA bases [24]. Besides, the probability of H-bond formation during DNA 3D motion is decreased as the open bases are required to align appropriately to form the bond.
Effective Hamiltonian and free energy. The small number of proteins compared to the number of base pairs allows us to reduce (1) to an effective mean-field type Hamiltonian. For that purpose we eliminate consequently \( J_{ij} \) and \( p_i \) variables while calculating the partition function \( Z = \text{Tr}_{\{p,\sigma\}} e^{-\beta H} \), where the trace means summing up over the corresponding variables. Taking the trace over \( J_{ij} \)'s [16] we arrive at the partition function \( Z \propto \text{Tr}_{\{p,\sigma\}} e^{-\beta H'} \) with the following effective Hamiltonian:

\[
H' = -g \sum_{i=1}^{N-1} \sigma_i \sigma_{i+1} - \gamma' \sum_{i<j} p_i p_j \sigma_i \sigma_j - \lambda \sum_{i<j} p_i p_j - h \sum_{i=1}^{N} \sigma_i - \epsilon \sum_{i=1}^{N} p_i. \tag{2}
\]

Here \( \gamma' = (c/N) \sinh \beta \gamma \) and \( \lambda = (c/N)(\cosh \beta \gamma - 1) \). The Hamiltonian (2) describes a system consisting of two interacting subsystems, DNA and proteins. Different time scales and different temperatures for the two subsystems may lead to novel phenomena [22]. However, here we assume that the DNA and proteins are in contact with the same heat bath at temperature \( T \).

For the case of strong binding energies \( \epsilon \gg \lambda, \gamma' \), we can make a mean-field approximation and replace the \( p_i \)s by their mean values \( \langle p \rangle = e^{3\beta \epsilon}/(1 + e^{3\beta \epsilon}) \); the proposed model is then reduced to the following effective Hamiltonian:

\[
H_{\text{eff}} = -g \sum_{i=1}^{N-1} \sigma_i \sigma_{i+1} - \gamma'' \sum_{i<j} \sigma_i \sigma_j - h \sum_{i=1}^{N} \sigma_i, \tag{3}
\]

where \( \gamma'' = (c/N) \sinh \beta \gamma \cdot (e^{3\beta \epsilon}/(1 + e^{3\beta \epsilon}))^2 \) represents the effective coupling between base pairs mediated by proteins. Notice that we have neglected the effect caused by the presence of the persistence length \( l_0 \) that would require us to take into account only the terms for which one has \( |i - j| > l_0 \) as the correction would be of order \( O(l_0/N) \) and would go to zero in the thermodynamic limit. The coupling in (3) is similar to that of a synchronization model with small world coupling [23].

To calculate the partition function \( Z \propto \text{Tr}_\mu e^{-\beta H_{\text{eff}}} \) for the Hamiltonian (3), we use the relationship \( \sum_{i<j} \sigma_i \sigma_j = \frac{1}{2}(\sum_i \sigma_i)^2 - \frac{1}{2} N \), the Hubbard–Stratonovich transformation \( e^{(1/2) a(\sum_i \sigma_i)^2} = \int_{-\infty}^{+\infty} (d \mu) e^{-a(1/2)}(\mu)^2 + a \mu \sum_i \sigma_i \) and the expression for the partition function of the one-dimensional (1D) Ising model [3]. Then the partition function takes the form \( Z \propto \int_{-\infty}^{+\infty} d \mu e^{-\beta N f(\mu)} \) with the effective free energy \( f(\mu) \) given by

\[
f(\mu) = \frac{1}{2} b \mu^2 - \beta^{-1} \ln[\cosh \beta(h + b \mu) + \sqrt{\sinh^2 \beta(h + b \mu) + e^{-4\beta g}}]. \tag{4}
\]

Here \( b = c \cdot \sinh \beta \gamma \cdot (e^{3\beta \epsilon}/(1 + e^{3\beta \epsilon}))^2 \) and \( \mu \) is the order parameter for the denaturation process. For the double helix state with all base pairs bound, \( \mu = -1 \); for the completely denatured state, \( \mu = 1 \). The values of \( \mu \), which determine the state of the molecule, are obtained via \( f'(\mu) = 0 \) that leads to the equation

\[
\mu = \frac{\sinh \beta(h + b \mu)}{\sqrt{\sinh^2 \beta(h + b \mu) + e^{-4\beta g}}}. \tag{5}
\]

The model is an effective Ising model with 1D nearest-neighbor and global (all-to-all) interactions. It can be shown that the model goes through a phase transition provided \( \beta b e^{3\beta g} \geq 1 \). That gives the necessary condition for the model parameters, e.g., the
Protein-mediated loops and phase transition in nonthermal denaturation of DNA

Figure 1. Double helix fraction $\theta = (1 - \mu)/2$ versus the base pair binding energy $h$ for the parameters $g = 8.5$ kcal mol$^{-1}$, $\gamma = 0.02$ kcal mol$^{-1}$, $c = 10$, $\epsilon = 7.2$ kcal mol$^{-1}$ and $k_B T = 0.6$ kcal mol$^{-1}$. The first-order denaturation phase transition occurs at the critical value $h_c = 0$. The critical torque is $\tau_c = 1.6 k_B T$ for AT-rich and $\tau_c = 7 k_B T$ for GC-rich chains. The double helix (coil) becomes metastable for $h > 0$ ($h < 0$) as indicated by dashes.

In order to quantify the degree of denaturation we introduce the parameter $\theta = (1 - \mu)/2$ that is the fraction of bound base pairs. The parameter takes the value $\theta = 1$ for the double helix state and the value $\theta = 0$ for the denatured coiled state. The dependence of the double helix fraction $\theta$ on $h$ is presented in figure 1. There is a metastability in a range of the controlling external parameter $h$. This effect is illustrated in figure 2 where the free energy with two minima is presented. Notice that there is no phase transition if the proteins do not interact ($\gamma = 0$) and thus the protein-mediated loops are absent. These are the looping proteins which provide us with the long-range interactions that make it possible to obtain a phase transition for the effectively 1D lattice model.

Transition time. The kinetics of the denaturation transition can be treated via the Langevin equation $\dot{\mu} = -\Gamma (\partial f(\mu)/\partial \mu) + \xi(t)$, where $\Gamma$ defines the inverse relaxation time, $\xi(t)$ is the white noise satisfying the relation $\langle \xi(t)\xi(t') \rangle = D \delta(t - t')$ with the diffusion coefficient $D$ determined by the fluctuation-dissipation relation $D = 2\Gamma k_B T$. The corresponding Fokker–Planck equation (FPE) for the probability distribution function $P(\mu)$ of the order parameter $\mu$ is $\dot{P} = (\partial/\partial \mu) A(\mu) P + \frac{1}{2} D (\partial^2/\partial \mu^2) P$, where $A(\mu) = -\Gamma (\partial f(\mu)/\partial \mu)$. Making the transformations $P \rightarrow P e^{f(\mu)/D}$, $D \rightarrow D \Gamma$ and $t \rightarrow t/\Gamma$, we can rewrite the FPE as $\dot{P} = HP$ with the Hamiltonian $H = -\frac{1}{2} D (\partial^2/\partial \mu^2) + (1/2D) \Phi^2 + \frac{1}{2} (\partial \Phi/\partial \mu)$, where $\Phi(\mu) = -f'(\mu)$. One can exactly solve the FPE to obtain $P_t(\mu, \mu_0) = \ldots \ldots$
Protein-mediated loops and phase transition in nonthermal denaturation of DNA

Figure 2. Free energy versus the order parameter $\mu$ at the base pair binding energy value $h = -0.05$ kcal mol$^{-1}$ for the parameters $g = 8.5$ kcal mol$^{-1}$, $\gamma = 0.02$ kcal mol$^{-1}$, $c = 10$, $\epsilon = 7.2$ kcal mol$^{-1}$ and $k_BT = 0.6$ kcal mol$^{-1}$. The double helix is stable and the coil is metastable for $h < 0$.

Let us consider the transition from the left minimum of the free energy in figure 2, corresponding to the native double helix state of DNA, to the right minimum representing the denatured state at the critical value $h_c$. At this value, which corresponds to the first-order phase transition point, the free energy is a symmetric curve with two equal minima and the maximum located at $\mu = 0$. The transition time $\Omega^{-1}$ (the inverse transition frequency) is twice the time needed to achieve the top of barrier $\mu_{\text{max}}$ from the minimum $\mu_{\text{min}}$ which is obtained from $\lambda_1$:

$$\Omega^{-1} \simeq \frac{2\pi}{\Gamma} \frac{e^{\beta[f(\mu_{\text{max}}) - f(\mu_{\text{min}})]}}{\sqrt{f''(\mu_{\text{min}}) f''(\mu_{\text{max}})}}$$

This is a standard expression for the Kramers problem [26]. However there is a qualitative difference since the potential $f(\mu)$ itself depends on temperature. The behavior of the denaturation transition time versus temperature drastically differs from the conventional Arrhenius case. Although the transition time first decreases at very low temperatures (frozen DNA) it begins to increase at high enough (physiological) temperatures. The reason is that the second derivative present in the denominator of equation (6) at the point $\mu_{\text{max}} = 0$ diverges since $f''(0) = b(1 - \beta be^{2\beta g})$ and $\beta be^{2\beta g} \to 1 (f''(0) \to 0)$ at the critical temperature defined by the above mentioned necessary condition of denaturation. For the set of parameters given in figures 1 and 2, the transition time is $\Omega^{-1} = 2.35 \times 10^{-5}\Gamma^{-1}$.

doi:10.1088/1742-5468/2011/01/P01005
The kinetics of pH-driven denaturation of DNA was studied experimentally in [27], where the transition time for single molecule denaturation was estimated to be of the order of 1–10 s. Taking these values we find the inverse relaxation time $\Gamma$ to be of the order of $10^{-6}$ Hz. However we believe that modern measurements in experiments with single molecules are needed to find precise values of the quantities.

**Conclusions.** In summary, we have introduced and studied a model of nonthermal denaturation of DNA that can be induced by chemical factors, such as pH value or salt concentration, or by externally applied mechanical forces and torques (as an example we considered the case of torque-induced denaturation) in the presence of protein-mediated loops. The model accounts for proteins that bind to the DNA molecule. The bound proteins are then allowed to interact in a random way with each other thus creating the loops. We have found a first-order denaturation phase transition that is caused by the looping proteins—the proteins that connect base pairs that are at a distance along the chain. The model possesses a metastability region provided that the necessary and sufficient conditions are satisfied. The kinetics of the denaturation phase transition was described by a stochastic dynamics for the order parameter that is, in principle, exactly solvable. However we have been mainly interested in obtaining the transition rate in the vicinity of the first-order phase transition. It has the standard Kramers form with the associated potential being temperature-dependent. This leads to deviation from the Arrhenius law at physiological temperatures. In particular, the transition time becomes extremely large when the temperature approaches its critical value that is defined by the necessary condition for the denaturation phase transition. The DNA denaturation kinetics considered here can be extended spatially to describe a front propagation process in the presence of protein-mediated loops and the noise that corresponds to the *in vivo* case.

Finally, we have revealed a new purpose of the protein-mediated looping, that is to facilitate *in vivo* denaturation of DNA needed to take it to the next transcription stage. The model also describes the formation of multiple loops via dynamical (fluctuational) linking between looping proteins, that is essential in cellular biological processes such as pre-mRNA splicing [28] and the phenomenon of genomic plasticity [29]. It can mimic, e.g., the co-evolutionary networks of splicing *cis*-regulatory elements [30] having the loops to splice out introns thus defining the exons within the DNA molecule. The presented theory can be applied in studies of the above described *in vivo* processes as well as for description of *in vitro* experiments with single DNA molecules. Yet another application of this or a generalized statistical mechanics model would be an investigation of dynamic genome architecture in eukaryotic cells [31]. In general, the presented approach can be extended to continue research on protein–DNA interaction networks where DNA looping mediated by proteins controls the balance between robustness and sensitivity of gene expression [32].

**Acknowledgments**

We thank A E Allahverdyan, D Mukamel, E I Shakhnovich, and M C Williams for comments and discussions. This work was supported by the National Science Council in Taiwan under Grant Nos NSC 96-2911-M 001-003-MY3, NSC 98-2811-M-001-066, and NSC 99-2811-M-001-060, and by the National Center for Theoretical Sciences in Taipei, Taiwan.

doi:10.1088/1742-5468/2011/01/P01005
References

[1] Watson J D et al, 2004 Molecular Biology of the Gene (Menlo Park, CA: Benjamin/Cummings)
[2] Wartell R M and Benight A S, 1985 Phys. Rep. 126 67
[3] Lavis D A and Bell G M, 1999 Statistical Mechanics of Lattice Systems vol 1 (Berlin: Springer) p 57
[4] Poland D and Scheraga H A, 1966 J. Chem. Phys. 45 1456
Poland D and Scheraga H A, 1966 J. Chem. Phys. 45 1464
[5] Peyrard M and Bishop A R, 1989 Phys. Rev. Lett. 62 2755
[6] Weber G et al, 2006 Nat. Phys. 2 55
[7] Strick T R et al, 1998 Biophys. J. 74 2016
Strick T R et al, 1999 Physica A 263 392
[8] Bryant Z et al, 2003 Nature 424 338
[9] Strick T R et al, 1998 Biophys. J. 74 2016
[10] Marko J F, 2007 Phys. Rev. E 76 021926
[11] Léger J F et al, 1999 Phys. Rev. Lett. 83 1066
[12] Cocco S and Monasson R, 1999 Nature Struct. Mol. Biol. 14 264
[13] Gore J et al, 2006 Nature 442 836
[14] Cocco S and Monasson R, 1999 Nature Struct. Mol. Biol. 11 1092
[15] Harada Y et al, 2001 Nature 409 113
[16] Marko J F, 2005 Multiple aspects of DNA and RNA: From Biophysics to Bioinformatics (Les Houches Session LXXII) (Amsterdam: Elsevier) p 211