Can re-entrance be observed in force-induced transitions?

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Abstract - A large conformational change in the reaction co-ordinate and the role of the solvent in the formation of base pairing are combined to settle a long-standing issue, i.e. prediction of re-entrance in the force-induced transition of DNA. A direct way to observe the re-entrance, i.e. a strand that goes to the closed state from the open state and again to the open state with increasing temperature, appears difficult to be achieved in the laboratory. An experimental protocol (in direct way) in the constant force ensemble is being proposed for the first time that will enable the observation of the re-entrance behavior in the force-temperature plane. Our exact results for a small oligonucleotide that forms a hairpin structure provide the evidence that re-entrance can be observed.

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It took almost four decades to realize that unzipping of double-stranded DNA (dsDNA) may be achieved with a force applied solely at one end instead of varying the temperature or pH of the solvent [1–5]. This became possible only because of recent advancements in experimental techniques (optical tweezers, atomic force microscope, magnetic tweezers etc.) which provided important information about the elastic, structural and functional properties of DNA [6–12]. Furthermore, these experiments elucidated the mechanism involved in crucial processes like transcription and replication [1,2,13–15]. Consequently by using force as a thermodynamic variable, many theoretical and numerical studies were done in establishing a wealth of definite falsifiable predictions [3,5,16–19]. Among these, a very important role was played by simple models which are amenable to analytical solution or very accurate numerical treatments [3,16,18]. Such models, despite their simplicity, have been proved to be quite predictive and provided many important information about the cellular processes. One of the most important predictions is the existence of a re-entrance behavior [16–18] in the force-temperature plane in the low-temperature region. Notably now the prediction of re-entrance is not only confined to DNA but also holds for other bio-polymers [20–25]. However, such re-entrance has so far remained elusive in the experimental studies.

One of the possible reasons for not observing re-entrance may be attributed to the fact that at low temperature most of the solvents would freeze, precluding any unzipping experiment. It is pertinent to mention here that the temperature ($T^*$) and force ($F^*$) used in these models [3, 16] are not in real units but in the reduced units which have been defined as

$$\frac{1}{T^*} = \frac{\epsilon}{k_B T},$$

$$\frac{F^* x}{T^*} = \frac{Fx}{k_B T},$$

where $\epsilon$ and $k_B$ are the effective base pairing energy and Boltzmann constant, respectively. Simplifying eqs. (1), (2) yields the relations $T = \frac{\epsilon}{k_B T^*}$ and $F = \epsilon F^*$ between real units and reduced units. Moreover, it is also now well established that the observed re-entrance is due to the ground-state entropy. Using phenomenological arguments [16,17], it has been shown that the critical force for the unzipping is $F^* = \epsilon + T^* s$ and the slope of the phase boundary (zipped-unzipped) at very low temperature in the force-temperature plane is $\frac{dF}{dT} = s$. Here $s$ is the entropy per base of the dsDNA. To observe re-entrance in vitro, $T^*$ as well as $s$ (ground-state entropy) should be large. However, in case of dsDNA or proteins, the ground-state entropy is not so large and thus such effect has not been seen.

In a recent controlled experiment, the effect of temperature on single-stranded DNA (ssDNA) has been studied [26]. It appears from this experiment that at a force higher than 12.5 pN, ssDNA attains the stretched
state and with the rise of temperature, there is an abrupt decrease in extension. This decrease is not due to the formation of hairpins because the solvent glyoxal (used in the experiment) disrupts the formation of hairpins [27]. Known polymer-based models could not provide the explanation for the observed decrease in the extension [26]. Kumar and Mishra developed a lattice model of ssDNA shown in fig. 1 with suitable constraints to show that this decrease may be an entropic effect [28]. As a consequence of this, the phase diagram obtained in the force-temperature plane (fig. 2) for this model also showed a re-entrance at low temperatures.

These important studies may facilitate the experimental design where re-entrance could be observed [26–28]. The important inferences that can be drawn from the above studies pertain to: i) The ideal candidate for such experiments should be a single-stranded oligonucleotide (a few bases of both ends are complimentary to each other) which forms a hairpin structure consisting of a stem (A-T or C-G) and a loop (C or G for A-T and A or T for C-G) [29,30]. In this case the reaction coordinate (end-to-end distance) will exhibit a large conformational change indicating the formation/disruption of the hairpin in comparison to λ-phase DNA. Moreover, the ground-state entropy of the system will be large due to the increased contribution from the loop. ii) The reduced temperature of the system may be decreased or increased by changing the concentration of glyoxal in the solvent, keeping the real temperature fixed [26,27]. It is pertinent to mention here that the solvent glyoxal used in the above experiments reacts with DNA. It introduces an additional ring to the G-base (to form a tricyclic compound, i.e. glyoxaldG), thereby sterically preventing the G-C base pair re-annealing [31–33]. Therefore, it may not be an ideal solvent to use as a denaturating solvent to observe the re-entrance.

Thermal melting of DNA occurs at high temperature (80–90°C). Since re-entrance has been considered as a low-temperature phenomenon, it is essential that the melting temperature of the system should be low. There are many solvents which can be used as denaturating agents (e.g. urea, formamide, formaldehyde, ethidium bromide etc.) to shift the DNA melting curve to lower temperatures [34–37]. In particular, formamide forms hydrogen bonds with the bases that replace the native ones and hence disrupts the base pairing of DNA. It has been seen experimentally that the melting temperature decreases linearly with the concentration. The relation which has has been used quite frequently to determine the melting temperature is
\[
T_m = 81.5 + 16.6 \log M + 41(xG + xC) - 500/L - 0.62f, \tag{3}
\]
where \(M\) is the molar concentration of monovalent cations, \(xG\) and \(xC\) are the mole fractions of G and C in the oligo, \(L\) is the shortest strand in the stem and \(f\) is the molar concentration of formamide [34,38]. In fact each % of change in concentration reduces the melting temperature by 0.6 degrees. The major advantage of this equation is that it includes the adjustment of salt and formamide. Moreover, for the molecular beacon, the melting temperature is about 40°C which can further be lowered by changing the loop length [29,30]. However, for a given loop length, by adjusting formamide concentration, the
melting temperature may be brought well below the room temperature.

The phase diagram presented in fig. 2 is in the reduced unit which shows a peak in force $F^*$ at a temperature equal to $T^* = 0.09$. To observe the re-entrance at a fixed force, say $F^* = 0.09$, the direct way is to follow the path $I$, $II$ and $III$ by varying the temperature. If we take base pairing energy equal to $0.19810^{-19}$ joule per base, then melting takes place at 313 K. Values of $T_1$ (open to closed) and $T_2$ (closed to open) are 50 K and 175 K, respectively. Such a low temperature and its variation over a large interval are difficult to achieve in vitro. In order to observe the re-entrance in vitro, we suggest to adopt an indirect way ($I'$, $II$ and $III$), which we shall discuss below, where by varying the solvent concentration one can go from the open ($I'$) to the closed ($II$) state, keeping the real temperature fixed.

In the following, we propose the experimental protocol, which may be used for the detection for re-entrance. The experimental setup used by Danilowicz et al. [26] is the starting point but instead of λ-DNA, we propose to use molecular beacons in the presence of formamide that form a hairpin structure [29,30]. From polymer theory, we know that a polymer chain will be in either a closed state (hairpin) or a swollen state (coil) depending on the temperature [39]. In the closed state (low temperatures) the average end-to-end distance $\langle R \rangle$ will be nearly equal to zero. At high temperatures, $\langle R \rangle$ scales as $N^\nu$ with $\nu = 3/(d+2)$, where $N$ is the length of the chain. It is worthwhile to note that in the observed experiment by varying the force, the polymer chain acquires the conformation of a stretched state. Hence force induces a new “stretched state” which is otherwise not accessible and with the rise of temperature, system attains the extended state.

In the presence of formamide, molecular beacons will attain a stretched state at temperature much lower than 40°C as observed in the absence of formamide [29,30]. Since in this region (stretched state) the stretching force increases with temperature (upper line shown by solid circles in fig. 2), the required force to keep the chain in the stretched state will also be less than 12.5 pN as seen in the experiment. If one now reduces the concentration of formamide at that temperature and force, there will be reannealing of hydrogen bonds associated with complementary end bases of the molecular beacon. This corresponds to the increase in the effective base pairing energy, and thus the reduced temperature and force of the system will decrease in order to keep the real temperature and force fixed. Since the applied force is not sufficient enough to keep the molecular beacon in the stretched state, the reduction in formamide concentration will drive the system into the closed (hairpin) state at the same temperature. In order to have higher closing rate (probability of forming the hairpin), we propose to use a stem of C-G and a loop made up of T. Since the rate of closing is much higher than the rate of opening (probability of opening), this will lead to the formation of base pairing resulting in a hairpin structure [29,30]. If so, then there will be an abrupt decrease in extension, i.e. reaction co-ordinate will approach to zero. Now with the rise in the temperature the system will again attain the open state. It means that in vitro, one would observe the system going to the closed state (hairpin) from the open (stretched) and again to the open (extended) state, i.e. $I'$, $II$ and $III$ instead of $I$, $II$ and $III$ with increasing real temperature.

In order to support the expected outcome of the proposed experiment, we adopt a more realistic model of DNA [19,28,40], which may be defined in any dimension. It was shown that the model takes care of important shortcomings of the known model for ssDNA and incorporates some additional features such as the excluded-volume effect, the directional nature of hydrogen bonds and the most important configurational entropy which gives rise the existence of stretched state. In this model, we consider a self-avoiding walk of $N$ steps ($N + 1$ vertices) on a cubic lattice (3D). A step of the walk represents a monomer of the chain. To each monomer a base is attached which has a direction associated with it. The first $n (= 3)$ bases of the walk represent nucleotide C and the last $n$ bases the complementary nucleotide G which forms a stem. The remaining $N - 2n = m$ bases constitute a loop of one type of nucleotides (T) which do not participate in pairing. The repulsion between monomers at short distances (i.e. excluded volume) is taken into account by the condition of self-avoidance. The pairing between bases can take place only when the bases at the two ends of the chain representing the complementary nucleotides approach on the neighboring lattice bonds with their directions pointing to each other and perpendicular to the lattice bonds they occupy (see fig. 3(a)). A base can pair at most with a complementary base. Energy is gained with the formation of each base pair. When the two bases representing complementary nucleotides approach on neighboring lattice bonds but with their directions not

![Fig. 3: (Color online) Schematic of configurations showing the formation (a) and non-formation (b) of base pairs.](38003-p3)
pointing to each other as shown in fig. 3(b), they do not pair and therefore there is no gain in energy. All possible conformations of ssDNA of $N$ bases mapped by the self-avoiding walks with the constraints specified above and having steps $N = 21$ on a cubic lattice (in 3D) have been exactly enumerated. The partition function of the system is found from the relation

$$Z_N = \sum_{p=0}^{n} \sum_{x=0}^{N} C_N(p, x) (e^{-\epsilon/k_B T})^p (e^{-F/k_B T})^x.$$  \hspace{1cm} (4)

Here, $C_N(p, x)$ is the total number of configurations corresponding to a walk of $N$ steps with a number $p$ of base pairs whose end points are at a distance $x$. $k_B$, $T$, $\epsilon$ and $F$ are the Boltzmann constant, the temperature of the system, the base pairing energy and the applied force, respectively. Following the method developed in [28,40], we obtained the phase diagram shown in fig. 2, where we set $\epsilon = 1$ and $k_B = 1$. The stacking energy which arises due to the hydrophobicity of the bases can be incorporated in the description. However, in this work we do not consider this interaction explicitly.

Here we focus ourselves on the study of the effect of formamide concentration (formation/disruption of hairpin).

In order to do so, we vary the base pairing interaction at constant temperature and force. $\epsilon = 0$ will correspond to an oligonucleotide in the presence of formamide at high concentration, and the average elongation due to the force can be found from the relations

$$\langle x \rangle = \sum x C(m, x) (e^{-\epsilon/k_B T})^p (e^{-F/k_B T})^x.$$  \hspace{1cm} (5)

The observed decrease in extension (reaction co-ordinate) with temperature shown in fig. 4 is solely an entropic effect. By setting $k_B = 1$, we get $F = F^* \epsilon$ and $T = T^* \epsilon$. The increase in $\epsilon$ means the decrease in formamide concentration and thereby a decrease in the value of the reduced temperature and of the applied force. If the applied force is high enough, the system will remain in the stretched state. But if it is in proximity of the upper line, an increase in $\epsilon$ (reduction in concentration of formamide) will lead to the base pairing among end bases in terms of formation of the hairpin. This indeed we find in the extension-temperature curve shown in fig. 4, where the decrease in extension approaches zero (showing the formation of the hairpin) at a particular base pairing interaction $\epsilon = 1.3$. If we increase temperature again, the system attains the open state as predicted by the force-temperature diagram shown in fig. 2.

It was shown that the probability distribution curves $P(x)$ with $x$ gives important information about the cellular processes [19,22,40] and may be calculated by using the following relation:

$$P(x) = \frac{1}{Z_N(m, x)} \sum_{m} C_N(m, x) (e^{-\epsilon/k_B T})^p (e^{-F/k_B T})^x.$$  \hspace{1cm} (6)

In fig. 5, we have shown $P(x)$ for different values of temperature at fixed $F^* = 0.12$ and $\epsilon = 1.3$. The $x$-component of the distribution function gives information about the states of the oligonucleotide. At $T^* = 0.03$, the peak value is near to 1, indicating that system is in the stretched state. Increase in temperature ($T^* = 0.07$) shows the sudden shift of the peak value near to 0 which reflects the formation of the hairpin. Further rise in
temperature bring the system from the closed (hairpin) state to the open state. This clearly demonstrates that re-entrance can be observed at ambient conditions.

It may be noted that the present model is a coarse-grained one, therefore, a quantitative estimate of thermodynamic parameters is difficult to predict. Moreover, the effects of salt concentration, pH of the solvent and other experimental parameters have been generally ignored in the description of coarse-grained models. The model can be parametrized [41], but there is not enough data so that the link among concentration of formamide, base pairing energy and a force can be established. This requires additional experimental and numerical works at this moment to study the effect of force in the presence of different solvents.

By combining the knowledge accumulated in different contexts e.g. kinetic of molecular beacons, role of solvent formamide, effect of temperature in constant-force ensemble, formation of hairpin kind of structure in DNA etc., we proposed an alternative way that will help in observing the re-entrance behavior in bio-polymeric systems at ambient conditions. The exact solution of the more realistic model of ssDNA supported by probability distribution analysis, renders an unequivocal support for the proposed setup.

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REFERENCES

[1] Boekelmann U., Essevaz-Roulet B. and Heslot F., Phys. Rev. Lett., 79 (1997) 4489.
[2] Boekelmann U., Essevaz-Roulet B. and Heslot F., Phys. Rev. E, 58 (1998) 2386.
[3] Bhattacharjee S. M., J. Phys. A, 33 (2000) L423 (cond-mat/9912297).
[4] Sebastian K., Phys. Rev. E, 62 (2000) 1128.
[5] Lubensky D. K. and Nelson D. R., Phys. Rev. Lett., 85 (2000) 1572.
[6] Smith S. B., Finzi L. and Bustamante C., Science, 258 (1992) 1122.
[7] Smith S. B., Cui Y. and Bustamante C., Science, 271 (1996) 795.
[8] Cluzel P. et al., Science, 271 (1996) 792.
[9] Wang M. D. et al., Biophys. J., 72 (1997) 1335.
[10] Rief M., Clausen-Schaumann H. and Gaub H. E., Nat. Struct. Biol., 6 (1999) 346.
[11] Bustamante C., Bryant Z. and Smith S. B., Nature, 42 (2003) 423.
[12] Bryant Z. et al., Nature, 424 (2003) 338.
[13] Lee G. E., Chrisey L. A. and Cotton R. J., Science, 266 (1994) 771.
[14] Danilowicz C. et al., Phys. Rev. Lett., 93 (2004) 078101.
[15] Danilowicz C. et al., Proc. Natl. Acad. Sci., U.S.A., 100 (2003) 1694.
[16] Marenduzzo D. et al., Phys. Rev. Lett., 88 (2002) 028102; Zhou H. J. et al., Phys. Rev. Lett., 97 (2006) 158302.
[17] Marenduzzo D., Trovato A. and Maritan A., Phys. Rev. E, 64 (2001) 031901.
[18] Kapri R., Bhattacharjee S. M. and Seno F., Phys. Rev. Lett., 93 (2004) 248102; Kumar S., Giri D. and Bhattacharjee S. M., Phys. Rev. E, 71 (2005) 051804.
[19] Giri D. and Kumar S., Phys. Rev. E, 73 (2006) 050903(R); Kumar S. and Singh Y., J. Phys A: Math. Gen., 26 (1993) L987.
[20] Marenduzzo D. et al., Phys. Rev. Lett., 90 (2003) 088301.
[21] Mishra P. K., Kumar S. and Singh Y., Europhys. Lett., 69 (2005) 102.
[22] Kumar S. and Giri D., Phys. Rev. Lett., 98 (2007) 048101.
[23] Kumar S. et al., Phys. Rev. E, 98 (2007) 1248101.
[24] Straeten E. V. and Naudts J., EPL, 81 (2008) 28007.
[25] Hanke A., Ochoa M. G. and Metzner R., Phys. Rev. Lett., 100 (2008) 018106.
[26] Danilowicz C. et al., Phys. Rev. E, 75 (2007) 030902(R).
[27] Desinges M. N. et al., Phys. Rev. Lett., 89 (2002) 248102.
[28] Kumar S. and Mishra G., Phys. Rev. E, 78 (2008) 011097.
[29] Bonnet G., Krichevsky O. and Libchaber A., Proc. Natl. Acad. Sci., U.S.A., 95 (1998) 8602.
[30] Goddard N. L., Bonnet G., Krichevsky O. and Libchaber A., Phys. Rev. Lett., 85 (2000) 2400.
[31] Broude N. E. and Budowsky E. I., Biochim. Biophys. Acta, 254 (1973) 380.
[32] Kasai H., Tanaka N. I. and Fukada S., Carcinogenesis, 19 (1998) 1459.
[33] Shokri L., McCauley M. J., Rouzina I. and Williams M. C., Biophys. J., 95 (2008) 1248.
[34] McConaughy B. L., Laird C. D. and McCarthy B. J., Biochemistry, 8 (1969) 3289.
[35] Hutton J. M., Nucl. Acids, 4 (1997) 1539.
[36] Bhattacharyya A. J. and Feingold, Talanta, 55 (2001) 943.
[37] Sadhu S. and Dutta S., J. Biosci., 6 (1984) 817.
[38] Howley P. M., Israel M. A., Law M.-F. and Martín M. A., J. Biol. Chem., 254 (1979) 4876.
[39] de Gennes P. G., Scaling Concepts in Polymer Physics (Cornell University Press, Ithaca) 1979; Kumar S. and Singh Y., Phys. Rev. A, 42 (1990) 7151.
[40] Kumar S., Giri D. and Singh Y., Europhys. Lett., 70 (2005) 15.
[41] Everaers R., Kumar S. and Simm C., Phys. Rev. E, 75 (2007) 041918.