Inhibitory effect of DNA supercoiling on DNA knotting.

Julien Dorier[1], Yannis Burnier[2] and Andrzej Stasiak[1*]
[1] Center for Integrative Genomics, University of Lausanne,
CH-1015 Lausanne, Switzerland
[2] Department of Physics, University of Bielefeld, D-33615 Bielefeld, Germany.

Abstract : Using numerical simulations we investigated the effect of DNA supercoiling on the topological equilibrium of DNA molecules. We showed that under the steady state conditions that maintain the same effective deficit of the linking number in unknotted and knotted DNA molecules the topological equilibrium results in a much smaller fraction of knots than in the case of torsionally relaxed DNA molecules. Based on these results we propose that one of the important functions of DNA supercoiling is to reduce formation of DNA knots. (This paper is a shorter, revised version of the article [1]. For the description of methods and more details please see the Ref. [1]).

1 Introduction

DNA replication, transcription and recombination are greatly facilitated by type II DNA topoisomerases that catalyze passages of one double-helical region through another [2, 3]. While the possibility of passing DNA segments through each other is generally beneficial, it may lead to the creation of DNA knots that are deleterious for living cells if not removed efficiently [4]. In 1997 a seminal paper demonstrated that topoisomerase II-mediated strand passages occur in a selective way that greatly reduces the formation of knots [5]. Since then many experimental, theoretical and simulation approaches addressed the question of how topoisomerases select intermolecular passages that preferentially lead to unknotting rather than to knotting of randomly fluctuating DNA molecules [5, 6, 7, 8, 9, 10]. In those studies, the circular DNA that was used or modeled as a substrate of DNA topoisomerases was torsionally relaxed. However, in living cells the DNA is rarely torsionally relaxed. In bacterial cells the DNA is negatively supercoiled by the action of DNA gyrase while, in eukaryotic cells, ongoing transcription or replication expose the DNA to torsional stress [11]. We, therefore, have analyzed the effect of DNA supercoiling on DNA knotting. Our numerical simulations reveal that DNA supercoiling inhibits intramolecular passages leading to DNA knotting.

If freely fluctuating, long, circular DNA molecules were permitted to undergo random intermolecular strand passages, they would be very frequently knotted [12, 13, 14]. However, when circular DNA molecules are isolated from bacteria, they are rarely knotted unless the host strain is defective in DNA gyrase [15]. This observation suggested that DNA supercoiling opposes DNA knotting. Though, in 1999 a simulation study that specifically addressed the effect of supercoiling on DNA knotting led to the conclusion that negative supercoiling promotes DNA knotting [16]. According to that study, even very small, naturally supercoiled DNA plasmids (2.4 kb) were predicted to adopt highly knotted configurations *in vivo* if specialized type II DNA topoisomerases did not bring the knotting level below the so-called topological equilibrium [5]. Intrigued by the contradiction between the experimental and numerical results, we performed

*E-mail: andrzej.stasiak@unil.ch

simulation studies with the hope of resolving the contradiction. We have found that the earlier modeling study by Podtelezhnikov et al. [16] did not account correctly for the homeostatic mechanisms that maintain a quasi constant level of torsional stress in DNA. With a more correct accounting, the numerical simulations agree with the biochemical data and point out that DNA supercoiling inhibits DNA knotting.

In their numerical simulations Podtelezhnikov et al., considered a situation where the difference of the linking number (ΔLk) of negatively supercoiled DNA molecules, as compared to torsionally relaxed circular DNA molecules with the same size, is maintained at the same value irrespectively whether these molecules are unknotted or knotted. This assumption on the first sight may seem to correspond to the *in vivo* situation in bacterial cells that are equipped with homeostatic mechanisms that keep the ΔLk of circular supercoiled DNA molecules with a given size nearly constant. So for example, if a given plasmid undergoes replication, the newly replicated daughter molecules are at some point nicked but after their ligation the action of DNA gyrase reestablishes their original level of DNA supercoiling and brings the ΔLk of daughter DNA molecules to essentially the same ΔLk as mother molecule had before the replication. However, as we explain below, knotted and unknotted DNA molecules will not be maintained at the same ΔLk by homeostatic mechanisms controlling the level of DNA supercoiling in bacterial cells.

As far as we know, the interplay between DNA gyrase, Topo I and also Topo IV [17] results in the same level of torsional tension in knotted and unknotted DNA molecules. To reach similar level of torsional tension knotted and unknotted DNA molecules of the same size would need to have similar linking number difference as compared to torsionally relaxed knotted and unknotted DNA molecules of the same size. However, this does not mean that knotted and unknotted DNA molecules supercoiled to the same torsional tension have the same ΔLk, as this term by definition uses the linking number of torsionally relaxed unknotted DNA of the same size as the subtrahend, while the torsionally relaxed unknotted and knotted DNA molecules differ in their linking number. This results from the fact that the DNA linking number (Lk) is the sum of DNA twist (Tw) and writhe (Wr), Lk = Tw + Wr [11]. In torsionally relaxed DNA forming a knot, the twist of the DNA is the same as in torsionally relaxed unknotted DNA molecules. However, the writhe is different. Therefore, the equilibrium linking number of knotted DNA molecules differs from that of unknotted DNA molecules and the difference is equal to the average writhe of torsionally relaxed knotted DNA molecules [19]. Importantly, the average writhe of relaxed knotted DNA molecules is independent of their length and depends only on the knot type [19, 20, 21]. The writhe of relaxed knots was calculated earlier [19, 20, 21] and confirmed by Podtelezhnikov et al. [16]. Thus, for example, the equilibrium linking number (Lk_0) of a left-handed trefoil knot is decreased by ca. 3.42 compared to the equilibrium linking number of an unknotted DNA. As the consequence the ΔLk between supercoiled DNA knots and torsionally relaxed unknotted DNA molecule can be decomposed into two components: 1. the difference of the linking number between torsionally relaxed knot and unknot 2. the difference of the linking number of supercoiled knot and torsionally relaxed DNA knot. Only the first component can be maintained at the quasi-constant level by homeostatic mechanism controlling the torsional tension in the DNA. We call this first component as the effective linking number difference (ΔLke). For supercoiled DNA molecules that are unknotted or form achiral knots the ΔLke is equall to ΔLk. In our simulations we address the problem of topological equilibrium under conditions that modeled molecules can freely tunnel between different topological forms but all these forms keep the initial ΔLke.

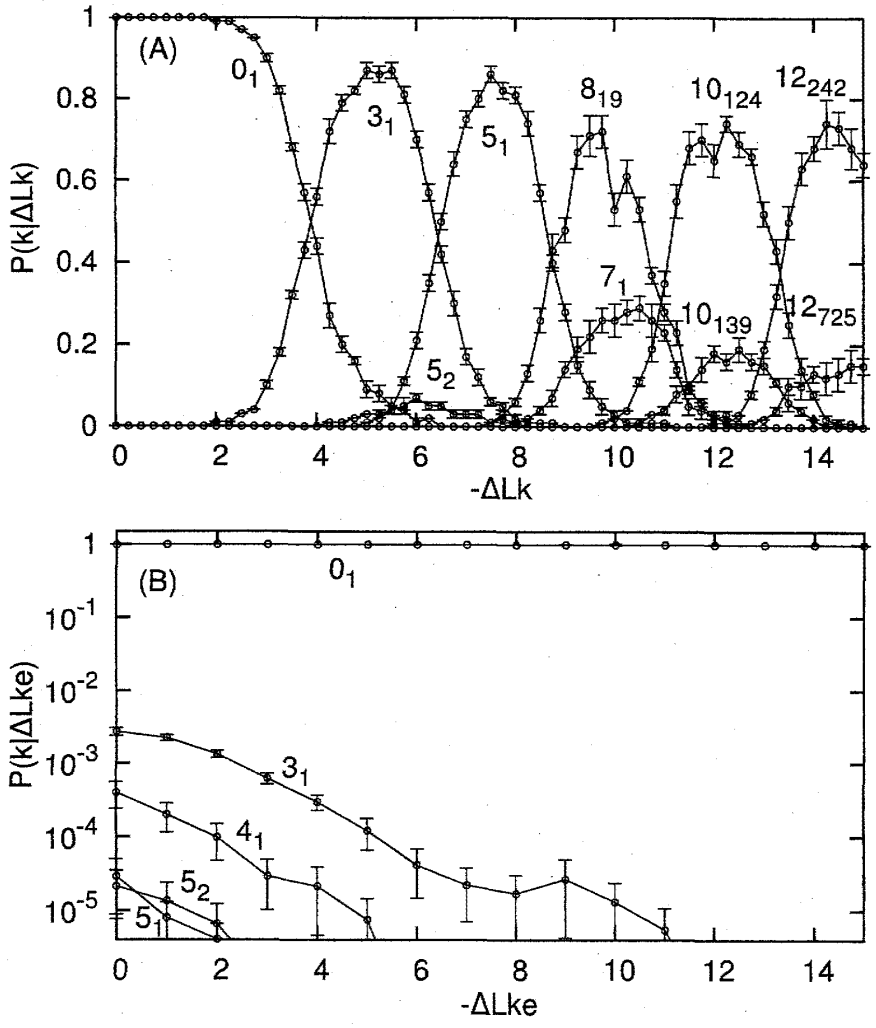

Figure 1: Comparison of probabilities of knotting in simulations that keep the DLk constant and those that maintain the same effective level of DNA supercoiling. (A) Conditional probability profiles of various knots obtained in numerical simulations where ΔLk was kept constant. (B). Conditional probability profiles of various knots obtained in numerical simulations where ΔLke was kept constant. The logarithmic scale shows that knotting is many orders of magnitude lower than knotting in simulations shown in panel A. Inset in B shows how changes of the effective diameter of modeled DNA affect the probability of trefoil knot formation for a given ΔLke.

2 Results and Discussion

Upon introducing the procedure that keeps the ΔLke unchanged upon change of the knot type we can calculate the topological equilibrium for various initial ΔLke. Figure 1 B shows that when ΔLke is kept constant, the frequency of knot formation decreased by several orders of magnitude when compared to the situation where simply ΔLk was kept constant as presented in Fig. 1 A. Importantly, only relatively simple knots were observed in a procedure that maintained the ΔLke constant and these were similar to those seen earlier with relaxed DNA [18, 22]. On other hand the very complex knots produced in simulations assuming conservation of ΔLk were not observed *in vivo* nor in experiments performed *in vitro*. It needs to be explained here why the condition of maintaining the same ΔLk leads to formation of knots, while the seemingly similar conditions of maintaining the same ΔLke opposes formation of knots. Left-handed torus knots such as those

observed in simulations performed by Podtelezhnikov et al., have strong negative writhe [16]. Therefore, covalently closed but torsionally relaxed DNA molecules forming these knots would have their linking number corresponding to this of torsionally stressed negatively supercoiled DNA molecules. For this reason in simulations performed under conditions of maintaining the same ΔLk, the modeled supercoiled unknotted DNA molecules got converted into left-handed torus knots as this permitted their torsional relaxation and thus lowered the elastic energy of these modeled DNA molecules. Why then, *in vivo* we do not observe such an elastic energy-driven conversion of supercoiled DNA into highly knotted forms? The answer lies in the fact that topoisomerase II-mediated passages from unknotted to knotted DNA molecules necessits the change of the linking number by two. This linking number change completely offsets the possible energetic gain of a hypothetical, unrealistic process where the DNA knotting could happen without the change of the linking number.

3 Concluding remarks

It is important to add here that the low probability of knotting, observed in our numerical simulation, simply reflected the thermodynamic equilibrium for DNA kept at constant ΔLke. We have assumed that cells maintain the steady state ΔLke value, which for a given size of DNA is the same for knotted and unknotted DNA molecules. We have also assumed that the molecules can freely tunnel between different topological states with the same ΔLke. In reality, just after topoisomerase-mediated passages the linking number difference between unknotted and knotted DNA molecules will amount to +2 or -2 (depending on the direction of the passage) and it will take some time till the homeostatic mechanisms involving cellular topoisomerases will reestablish similar ΔLke as before the passage. For our simulations we also assumed that the passages are not ATP driven and therefore acceptance or rejection of a configuration with a given topological state would be simply dependent on the energy difference between configurations adopting different topologies. It is a qualitatively different situation to experiments described by Rybenkov et al. where the type II DNA topoisomerases were using the energy of ATP hydrolysis to decrease the knotting level below the topological equilibrium [23]. But our results indicate that the energy of ATP hydrolysis needed to keep the DNA at steady state, nearly constant level of DNA supercoiling can be also used to lower the frequency of DNA knotting. Over the last ten years, several studies addressed the question of how DNA topoisomerases maintain the DNA knotting level below the topological equilibrium that would be obtained if topoisomerase-mediated intersegmental passages were occurring at random [8, 9, 10, 24]. These studies, in large part, explored non-supercoiled DNA since the original experiments were performed using non-supercoiled DNA molecules [5]. Although the experiments measuring the steady state catenation and knotting level in systems where gyrase supercoils DNA molecules and where Topo IV acts on them are more difficult than those performed with relaxed DNA molecules, they would be more likely to provide us with important information on the effect of DNA supercoiling on DNA decatenation and unknotting activity. Simulations reported here constitute a step in this direction.

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