Biomedical researchers regularly stretch and twist single DNA molecules in magnetic tweezer experiments. By making the molecule writhe into a plectoneme (ply) and plotting its load–extension curves, key DNA parameters, such as effective radius, can be estimated. Adding untangling enzymes (topoisomerases) to the DNA’s environment, their individual cuts are detected as jumps in extension.

Sufficient information is now known about the topoisomerases for us to make good idealizations about their kinematics and mechanics. The novelty of this paper is to study their actions in the context of accurate ply solutions from the theory of elastic rods. To do this, we define an extended rod-plus-tension system that allows us to determine the stored energies from areas in the conventional link versus writhe plane.

After a cut, the molecule relaxes dynamically to a new equilibrium state, and often there will be two or more alternative stable configurations onto which it might settle. Knowing the energy levels allows us to identify which states can and cannot be reached over the unstable mountain passes, and which of the accessible states offer the greatest energy relaxation. Strict energy bounds on behaviour are established.

This knowledge has medical value because topoisomerase inhibitors, lethal for cells, are used as antibiotics and in chemotherapy for cancer.

Keywords: supercoiling of DNA; single-molecule experiments; topoisomerase; plectoneme mechanics; magnetic tweezers

1. Introduction

Mechanical tests on natural double-stranded DNA (often written as dsDNA where confusion with a single strand might arise) are regularly performed in a magnetic tweezer experiment that allows a single molecule to be simultaneously stretched and twisted (figure 1). Other forms of loading include optical traps, and excellent reviews of this work are given by Bustamante et al. (2003) and Ritort (2006).

The controlled loading parameters are the (dead) tensile force, \( T \), and the (rigid) end rotation, our measure of the latter being the number of complete turns (\( n \)), namely the link, \( L_k \). Note that in this paper, the DNA is being treated

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primarily as an elastic rod with no reference to its internal helical structure; for this reason we do not need to distinguish between the link $L_k$ and the increment $\Delta L_k$, which is often employed in the literature. Under this loading, the experimentalist is able to measure the varying distance between the two ends of the molecule, $Z$, which provides a measure of its extension and deformation.

In a typical test, $T$ is held constant and the variation of $Z$ with $L_k$ is recorded, as shown in figure 1b. From the hat-shaped curves, it is easily deduced that under increasing link the molecule has formed a lateral plectoneme (ply), as illustrated in figure 1a. By fitting the experimental curves to theoretical results, Neukirch (2004) showed how we are able to estimate the effective radius and torsional stiffness of the molecule. In Neukirch’s work, and throughout this paper, the DNA is modelled theoretically as a thin elastic rod, because on the scales involved in the experiment the internal double-helical structure of the DNA is usually irrelevant; this rod is assumed to be uniform along its length, with no (sequence-dependent) variation of its bending or torsional stiffnesses. Such a classical, deterministic study of the problem provides a firm framework against which more detailed thermodynamic analysis can be viewed. It also puts into sharp focus some energy questions that do not seem to have been correctly addressed in the literature.

A second use for these tests is to study the cutting action of the topoisomerase enzymes. These play a vital role in a cell, cutting the DNA, as necessary, to prevent unwanted tangling and knotting of the long molecule (Calladine et al. 2004; Travers & Thompson 2004; Bates & Maxwell 2005). When, for example, a type 2 topoisomerase (subsequently written as Topo 2) is added to the environment of a ply in a tweezer experiment, sudden jumps in $Z$ (of approximately 90 nm) are observed, corresponding to the release of 2 units of $L_k$, as shown in figure 1c. This observation of individual topoisomerase cycles in real time permits an accurate characterization of the enzyme’s biochemical cycle.
The consequential improvement in our knowledge has real medical value; blocking the cycle of topoisomerases is lethal for the cell, and topoisomerase inhibitors are extensively used both as antibiotics and in chemotherapy for anti-cancer treatment.

This paper is directed towards a more complete understanding of this Topo 2 relaxation process in the context of accurate numerical solutions from the theory of elastic rods by Swigon (1999), Coleman & Swigon (2002) and Neukirch (2004). The two graphs that we use specifically for our illustrations are the following: in figures 4, 9 and 11, a graph from Swigon (1999) that is useful at low values of the link and in figures 5–7, an unpublished graph at higher link kindly supplied by Sébastien Neukirch, whose theoretical approach is sketched in Neukirch (2004). Other relevant work on the ply mechanics of elastic rods should be noted: Coleman & Swigon (2000) obtained accurate results for DNA plasmids (closed loops); Thompson et al. (2002) studied a generalized loaded ply; and Clauvelin et al. (2007) made an approximate analytical analysis.

2. Kinematics of the topoisomerase

There are two basic types of topoisomerase, type 1 and type 2. The first, Topo 1, operates on an isolated segment of the dsDNA molecule. It cuts this segment (strictly it cuts just one of the backbone helices), allows one (or more) rotations of 360° and rejoins it. This changes the link, L_k, by ±1 (or a multiple thereof). Examples of Topo 1 are topoisomerase I, reverse gyrase, topoisomerase III and topoisomerase V. Here, the roman numerals simply refer to the historical order of discovery.

By contrast, Topo 2 operates on two segments of DNA that are in mutual contact (or at least very close to one another). It cuts one of the segments, passes the second segment through the gap and rejoins the first segment, with no rotation. An illustration of how a Topo 2 might perform these tasks is shown in figure 2, adapted from the paper by Wang (1998). Note in passing that, in this model, the enzyme requires the consumption of two adenosine tri-phosphate (ATP) molecules to

Figure 2. Notional action of a Topo 2 adapted from Wang (1998). Note that the G-segment forms a gate, through which the T-segment is transported.
perform its cycle; we recall that the hydrolysis of ATP fuels nearly all enzymatic activity. The described action changes the link, Lk, by ±2. Examples of Topo 2 are *topoisomerase II*, *gyrase*, *topoisomerase IV* and *topoisomerase VI*.

So far in this paper, we have discussed only the kinematics (the geometry) of the processes; in the next section we will consider also the mechanics, and in particular the energy exchanges.

### 3. Idealized mechanical operations

In applying the concepts of applied mechanics, and in particular the theory of elastic stability (Thompson & Hunt 1973), to the cutting action of the topoisomerases, we are confronted with a wide variety of these enzymes that differ in their precise mode of operation, even within the two broad types outlined above. For some, the mechanics of the operation are reasonably well understood; for others there may be considerable uncertainty. So in order to proceed with this paper’s demonstration of mechanical principles, we shall define for each type an idealized but plausible mode of operation, as described below.

For the Topo 1 operation, we might imagine ourselves cutting a thin silicone rubber rod with scissors in the laboratory. Let us suppose that a colleague makes the cut as I grip the segment of rod with my fingers on either side. Holding one side still, I rotate the other side through 360° and glue the ends back together. To make the operation more precise, we must discuss the speed of the cutting process in relation to possible deformations of the molecule. Fortunately, the DNA will normally be in a relatively viscous environment, so it is reasonable to assume that the molecule does not move (appreciably) during the cutting process. Following Thompson (in press), we can adopt this as our idealized process, but any change of energy in real DNA may depend on the particular mode of operation of the cutting enzyme. For this reason, we shall leave the mechanics of Topo 1 for future study and concentrate in this paper on the mechanics of Topo 2, which allows much sharper definition. For completeness, however, we shall sketch the basic operation of Topo 1 in figure 6. We can finally draw attention to recent work on topoisomerase V, a type 1 enzyme, reported by Taneja et al. (2007); these authors show that with this enzyme the action is powered by the torque in the supercoiled DNA and is constrained by friction between protein and the DNA.

To discuss Topo 2 mechanics we will need three people for our thought experiment. We assume that at the beginning the two rod segments are not only touching one another geometrically but are also *pressing* one against the other mechanically; in a mechanical context a simple touch would be untypical. A colleague makes the cut as I grip one of the rod segments with my fingers on either side. Meanwhile, a second colleague who has been holding the second segment moves it through the gap. I then glue the ends of the cut segment back together again with no relative rotation. Because we are assuming the rods to be (arbitrarily) thin compared with the lengths of rod involved, this action has minimal effect on the molecule. So long as we keep hold of the rearranged segments, the system is unaware of any change, and importantly, its energy is unaltered by what we have done. If we finally release the segments, the lack of the earlier contact pressure means that the system will no longer be in
equilibrium, and a dynamical motion will ensue. Luckily, our knowledge of the starting energy will allow us to make predictions as to how and where the motion might end. This idealized Topo 2 action is well defined, and we can reasonably expect it to be close to reality. It is the primary topic of the rest of the paper.

We note again that Topo 2 is known to use the energy of ATP hydrolysis in its operation (Strick et al. 2002; Bates & Maxwell 2005; Maxwell et al. 2005). We discuss this more fully in a later section, but here we are implicitly assuming that this energy is just used to cut and rejoin the segment and in particular, does not impart any momentum to the molecule.

4. A little topology

Consider an inextensional rod of circular cross section, with length $L$ and radius $r$. To discuss torsion, we imagine parallel stripes drawn on the surface of the straight and unstressed rod parallel to its centre line. While it is (held) straight, let the rod be twisted uniformly by applying a rotation about the centre line, $\phi$, (in radians) to one end. The kinematic twist rate is then $\tau = \phi / L$, and is positive if the stripes form a right-handed helix. The total twist, $Tw$ (in turns), is defined as the integral of $\tau / 2\pi$ over $L$. When $\tau$ remains constant along the rod, as it does in this thought experiment and (despite all the self-contacts) in the ply solutions of Swigon (1999) and Neukirch (2004), the integration gives immediately

$$Tw = \frac{\tau L}{2\pi}.$$ (4.1)

Keeping the ends glued to in-line sliders, so that they can move towards each other without changing $\phi$, we shall discuss the rod’s spatial deformations (natural, or imposed by hand) by writing the number of complete turns as the link, $Lk = \phi / 2\pi$. As long as the rod remains straight (or even planar), we have $Lk = Tw$. However, once the rod adopts a fully three-dimensional form, the total twist changes and the two are related by the important topological result,

$$Lk = Tw + Wr.$$ (4.2)

Here the writhe, $Wr$, is a property of the shape of the rod’s centre line and can be calculated as the number of (signed) crossings in a view, averaged over all views. Loosely, it can be thought of as the degree to which a curve is ‘helix-like’.

An excellent account of equation (4.2) and the historical papers from which it derives (Călugăreanu 1959, 1961; White 1969; Fuller 1971, 1978) are given by Moffatt & Ricca (1992).

We should note that the concepts of link, twist and writhe, and the equation between them, are at their sharpest for a closed curve; however, they can be carried over, with care, to the magnetic tweezer experiment as outlined above (van der Heijden & Thompson 2000).

5. Laboratory experiments on ply formation

Before embarking on the theory, we start by looking at the experiments of Thompson & Champneys (1996) on a long elastic rod, made of silicone rubber, which is stretched and twisted in a rigid loading rig. This rig controls the extension, $Z$, (rather than the dead tension of the tweezer experiment) and the
link, Lk; the equilibrium states will be the same as in the tweezer test, but certain unstable solutions will be stabilized, making them observable. For a detailed review of the dead and rigid loading of elastic structural systems, including the concepts of internal and external stability, see Thompson (1979). The results of the tests (based on photographs of a specimen as $Z$ is decreased) are summarized in figure 3c–f. In this sequence, the link is held constant and the ends are brought slowly together by a quasi-static reduction of $Z$; during this process $T$ and $M$ will passively adjust their magnitudes.

Here, the length, $L$, is long in comparison with the radius, $r$, and also long in comparison with the deformation wavelengths. The rod is pulled by a tension $T$, which does work through the extension $Z$, and is twisted by an end moment $M$, which does work through the end rotation Lk. A small-deflection linear buckling analysis predicts that the straight ‘trivial’ form of the rod will become unstable (in the limit as $L/r \to \infty$) at a critical value of

$$M = 2\sqrt{(BT)},$$

(5.1)

where $B$ is the bending stiffness of the rod. Note that this formula, presented by Love (1927), does not involve the torsional stiffness of the rod, $C$.

At this critical condition, the rod is predicted to buckle, at small amplitude, into the helical form shown in figure 3b. This form was, however, never observed experimentally in our mechanical tests on rubber and metal specimens, and large-deflection nonlinear theory shows that the preferred deformation will be a localized one as shown. This type of spatial localization has been greatly illuminated by work employing the static–dynamic analogy (Hunt et al. 1989; Champneys & Thompson 1996; Champneys et al. 1997; Hunt 2006), where the localized form corresponds to a homoclinic orbit of an equivalent dynamical system. The equivalent dynamical system for a rod is a spinning top (with arc length of the rod corresponding to the time for the top), as observed by Kirchhoff. If the rod has a non-symmetric cross section (with two distinct

\[ \text{Figure 3. An experimentally observed sequence showing localization under rigid loading.} \]

\[ (a) \text{ The trivial unbuckled state, (b) the theoretical helical buckling of a long rod, (c,d) progressive localization, (e) self-contact after a fast dynamic jump, and (f) the steadily growing lateral ply.} \]
principal bending stiffnesses), then the equivalent top will have no rotational
symmetry; such a rod can exhibit spatial chaos (Mielke & Holmes 1988), while
the top can exhibit temporal chaos (van der Heijden & Thompson 2002).

In the rigid conditions of the experiment, the localization can be partially
observed under static conditions (figure 3c, d), until a sudden dynamic jump
takes the rod into the configuration of self-contact shown in figure 3e. This
subsequently grows into a lateral ply as the experiment progresses under slowly
derectly decreasing $Z$ (figure 3f). More comprehensive experiments are reported by Goss
et al. (2005).

6. The theory of ply formation

We must now outline the theoretical work on the buckling into a ply of a
stretched and twisted rod; this will then form the framework on which to analyse
the workings of the topoisomerase.

Some precise numerical solutions by Swigon (1999) for the buckling of a DNA
molecule under tension and torsion are shown in figure 4. For a constant and
dead tension, $T$, this graph shows the spatial equilibrium configurations of the
molecule, taking full account of all (frictionless) self-contacts, as the rigidly
imposed link, $L_k$, is slowly varied. As the measure of deformation, Swigon has
adopted the writhe, $W_r$, which increases continuously as the ply lengthens. The
rod is stable in its straight unbuckled trivial state with $W_r = 0$ up to the
subcritical bifurcation at $A$, from which a dynamic jump would carry the DNA
from $A$ to a state of self-contact at $E$, as illustrated by the horizontal arrow. Note
that on a plot of $L_k$ against $W_r$, the post-buckling path will not in general emerge
from the bifurcation point $A$ with zero slope (as it superficially appears to
do here, but not in figure 5, which has different parameters); this is because
$W_r$, akin to the end shortening of an Euler column, is orthogonal to the
buckling mode.

Figure 4. The buckling of a stretched and twisted rod by Swigon (1999), corresponding to a DNA
molecule of 1000 bps, of length $L = 3400 \, \text{Å}$, under a tension of $T = 0.33 \times 10^{-12} \, \text{N}$. 

Ignoring, for the moment, the physical jumping behaviour, we can focus on the post-buckling path that emerges from A. Note that a solid (or broken) line denotes a stable (or unstable) path under controlled Lk. Between A and B, we have an unstable falling path with no self-contact. Between B and D, we have a path with one self-contact at a point. As we move from D towards E, the path develops self-contact at two points, then at three points; the third central contact finally smears out into a continuous line of self-contact, namely a ply, with the first two contacts remaining nearby. Physical jumps under slowly varying Lk are indicated by horizontal lines, and sample computed shapes are displayed.

To calculate energies, we shall need to know the magnitude of the twist, Tw, at points on the equilibrium paths, and remembering the topological result (4.2) it is clear that this is given by the horizontal distance to a point from the 45° line on which Lk = Wr, as illustrated.

7. Successive relaxations

We suppose now that we have our magnetic tweezer specimen loaded up so that it is in equilibrium with a central ply. In particular we assume, for example, that it is at point A in figure 5. This figure is based on calculations by Neukirch (2004) and is essentially the same as figure 4, but extends to somewhat higher values of Lk. Parameters such as the slenderness ratio of the molecule and the ratio of its bending to torsional stiffness are, however, different, and we note that the initial slope of the post-buckling path is here quite clearly non-zero.

Making no change to the loading imposed by the tweezers, we now trace out what would happen if a Topo 2 were introduced to act on the specimen. We assume for simplicity of exposition that the cuts always induce relaxation rather than tightening of the ply; tightening would in fact be rather exceptional, especially at high Lk where the ply is very tightly wound. Where Topo 2 is likely
to make its cut, in the central region of the ply or at its ends, is not clear. This will obviously affect the dynamics of the relaxation process, but will not influence our following discussions.

Starting at A, a cut would decrease the link, \( L_k \), by 2, and the writhe, \( W_r \), by an approximately equal amount. The small deviation of \( W_r \) from 2 is entirely due to the finite size of the DNA cross section, and this small effect will not concern us in the present paper. So following the schematic curved black arrow, with \( \Delta L_k = \Delta W_r = -2 \), the molecule would immediately be displaced to point a where it is no longer in equilibrium. The molecule will now relax dynamically and settle onto one of the available stable equilibrium states. There are in fact two such states available, the ply solution at B and the straight trivial state at \( B^T \).

Without the energy considerations that we introduce in the following section, we are at the moment unable to say to which of these two states the system will settle. The fact that a looks close to B on this diagram is not a guide, and as we shall see, is misleading, as the two states have completely different deformations and different energies. This would be true, even if a and B were exactly superimposed in the link–writhe space; this point will be greatly clarified by figure 8.

If the molecule were to settle at B, a further cut, by the same or a different Topo 2, would take the system to b and a second uncertainty would arise about settling at C or \( C^T \). A further cut would give us an even bigger uncertainty (which is nevertheless amenable to the forthcoming energy techniques), because from c there are three competing stable solutions, \( D \), \( D^N \) and \( D^T \). The jumps located between A and D that we have described above are precisely the (seemingly random) jumps observed experimentally in figure 1c.

Although we are not pursuing Topo 1 in detail in this paper, we give, for completeness, its cutting response in figure 6; this can be compared directly with the behaviour of Topo 2, which we have just described in figure 5. Even though we have not made a complete study of the energy transition for Topo 1, we can identify the energy relaxations achieved in the steps using the equalities that we shall establish in §8.
Note that in figure 6, we give just a brief summary of the main response to a type 1 topoisomerase, without drawing attention to the alternative outcomes, etc. The shaded area shows the relaxation of stored energy achieved by passing from state F to state G using the concepts of the following section.

8. Energy concepts and link–writhe areas

The use of energy concepts for problems of this type was introduced by Thompson (in press). In that article, devoted exclusively to circular DNA plasmids rather than the magnetic tweezer test, it was shown that areas in the \((Lk, Wr)\) plane are related directly to changes in the energy of the system, and we shall now see that a similar result holds in the present situation if we carefully define an extended system for our energy studies. Then, for the tweezer experiment, we make a more complete investigation of all energy levels; in particular, by determining the energy levels of the unstable states, we are able to establish important energy bounds for the first time.

We consider, as our mechanical system, the DNA molecule and the dead tensile load with its associated magnetic energy field. This extended system is subjected to a generalized force, namely the torque, \(M\), provided by the magnets, acting through its corresponding generalized displacement, namely the turns of link, \(Lk\). The torque provided is just the kinematical twist rate of the molecule, \(\tau\), at the end of the rod (in radians per unit length of the rod’s centre line) multiplied by the torsional stiffness, \(C\). As already explained, the twist rate, \(\tau\), remains constant along any equilibrium configuration of the molecule. The twist rate is therefore related to the total twist, \(Tw\), by equation (4.1) and we have

\[
\text{generalized force } = M = C\tau = 2\pi CTw/L \quad (8.1)
\]

and

\[
\text{corresponding displacement } = 2\pi Lk. \quad (8.2)
\]

So, if the molecule were to follow the equilibrium path from the origin (in a virtual thought experiment) through stable and unstable states, with the load going up and down as needed to preserve equilibrium, the stored energy at any point on the path would be given by the integral along the equilibrium path

\[
V = (2\pi)^2 (C/L) \int Tw \, dLk. \quad (8.3)
\]

Although it looks superficially like an expression for just the twisting energy, the expression for \(V\) actually gives the total strain energy of the DNA (with contributions from both twisting and bending) plus the stored magnetic energy associated with the tension \(T\). So, within the factor \((2\pi)^2(C/L)\), the stored energy can be identified as areas in the \((Lk, Wr)\) plane bounded by the 45° line from which the \(Tw\) can be measured.

Using areas to give energies is a very useful visual tool, as we clearly demonstrate in this paper. It is often essential when using other theoreticians’ published graphs, because the original computed data are usually unavailable,
and it could be useful in analysing experimental results. However, a theoretician analysing a model of DNA would, of course, be able to compute the necessary energy levels directly within the computer modelling, using the basic expression for the stored energy

\[ V = \frac{1}{2} \int (B\kappa^2 + C\tau^2) \, ds - TZ. \] (8.4)

Here, the integration is over the length of the rod, \( \kappa \) is the resultant curvature of the rod and \( s \) is the arc length along the rod’s centre line. Note carefully that this \( V \), which we employ in this paper, includes the potential magnetic energy of the axial tension \( -TZ \). It does not include the (magnetic) energy of the end twisting moment, which we view separately as an external load acting on the rod-plus-tension system.

9. Energy balances during a cut and its relaxation

Using these area-energy rules, we can now study the cutting actions of Topo 2 more closely. In figure 7, we show the cut from A to a, which, we know, involves no change of energy with \( V(A) = V(a) \). So, when the molecule starts to move as it relaxes towards an equilibrium state at constant \( Lk \), we can check to see if its starting energy is sufficient to surmount the mountain pass at the unstable equilibrium state \( H \).

We regard each area (X, Y, Z, etc.) as a positive quantity, and would emphasize that, although indicated by two letters for clarity, there is only one unshaded area W extending round from \( BT \) to B. Introducing the notation, \( x \equiv (2\pi)^2 \frac{C}{L} \) (area X), etc, a little geometrical consideration reveals that

\[ V(B^T) = w + x, \] (9.1)
\[ V(H) = w + x + z, \] (9.2)
\[ V(B) = w + z \] (9.3)

and

\[ V(A) = V(a) = w + z + y. \] (9.4)
We note in particular that

\[ V(A) - V(H) = y - x, \]  

(9.5)

so, given that area X is clearly greater than area Y, we have \( V(A) < V(H) \).

Consequently, the cut from A cannot get over the pass and the stable trivial (straight) configuration, \( B^T \), is inaccessible; the motion will inevitably come to rest at B. We can, moreover, see that the reduction of energy achieved by the cut is given by

\[ V(A) - V(B) = y. \]  

(9.6)

Based on these equations, we can illustrate the energy surface of the system by the schematic drawing of figure 8. Here, the generalized coordinate \( q_i \) represents progress along the path while coordinate \( q_j \) represents displacements orthogonal to the path. In this figure, we can imagine a ball rolling on the surface; released at \( a \), it has insufficient energy to surmount the pass at \( H \), which would be needed if it were to settle at \( B^T \).

While thinking about this figure, we should emphasize another feature of our argument. In general, there are many deformation paths that the system can take, and there may be mountain passes on other equilibrium paths that are not drawn on figure 7. Indeed, Swigon (1999) uncovered a wide variety of different equilibrium paths emanating (for example) from higher branching points. However, it was shown (Swigon 1999; Tobias et al. 2000) that \( H \) (on the path from the lowest bifurcation point on the trivial path) is the lowest pass for the transition from \( B \) to \( B^T \). This follows from the fact that each configuration on the drawn path minimizes the energy \( V \) of equation (8.4) among all configurations with the same writhe (and hence the same \( L_k, Tw \) and torsional energy). This is illustrated nicely in figure 8 if we think of \( q_i \) as representing the writhe and \( q_j \) as the magnitude of an orthogonal deformation.

10. Derivation of energy bounds

It is clear, from energy considerations (and the tightness of the ply), that relaxation to the straight configuration cannot occur from a high-link start, and the area analysis of the previous section shows that it will become possible once area X is equal to area Y, as illustrated in figure 9.
This figure shows the highest starting point, at $L_k(A)$, for which the relaxation to $B^T$ is energetically possible. This is the upper bound for relaxation to the trivial solution, which we write as $L_{k_{UT}}$. It is only a bound because having enough energy to get over a mountain pass does not guarantee that the system will follow this route. It might be initially moving in a different direction (as in figure 8), and it will in any case be dissipating energy throughout its motion due to fluid viscosity, which at the scale of DNA is relatively high. All we can say is that the starting link, $L_k^T$, giving the first arrival at $B^T$, is constrained by the inequality

$$L_k^T \leq L_{k_{UT}}.$$  \hspace{1cm} (10.1)

The equal-energy condition is illustrated in the schematic diagram of figure 10. Here, in the absence of any energy dissipation, a ball released from $a$ has just enough energy to get to the mountain pass, $H$.

An additional bound arises when we look at the energy relaxation achieved. As in the previous section, we can see that if the cut from $L_{k_{UT}}$ settles at $B$, the relaxation of stored energy is given by area $Y$. Meanwhile if it settles to $B^T$ the energy relaxation achieved is reduced by $(x-z)$. So, for the first relaxation to a lower energy trivial state, from $L_k^E$, we can introduce a second inequality

$$L_k^E \leq L_{k_{UE}};$$  \hspace{1cm} (10.2)

where $L_{k_{UE}}$ is the starting point that makes area $X$ equal to area $Z$. This is akin to the Maxwell equal-energy criterion, which was originally conceived for stochastically driven thermodynamic processes under Brownian agitation. It has re-emerged in theoretical mechanics (Hunt et al. 1989; Hunt & Neto 1993; van der Heijden et al. 2002; Hunt 2006), where it is deeply associated with the development of localization in the static–dynamic analogy. In the present

Figure 9. The critical condition of equal areas. Here, because area $X = area \ Y$, the cut from $A$ has just enough energy to reach $H$ and pass over to the trivial solution $B^T$. This is the first possibility of relaxation to the straight configuration as the link of the starting point is decreased. Graph from Swigon (1999).
context it does, of course, only represent a bound, and there is no guarantee that the system will surmount the mountain pass even though there is a lower valley beyond.

11. Multiple outcomes at lower link

More complications are clearly about to arise (even before we reach the Maxwell condition) as our discussion heads down towards the emergence of a third alternative stable state at lower link (figure 11). These complications are easily sorted out using the energy principles that we have established in this paper, but their detailed description here would serve no purpose and could possibly even obscure the basic message.
So, we content ourselves with writing down the following energy values and making a few observations,

\[ V(B^T) = w + x + s, \]  \hfill (11.1)
\[ V(H) = w + x + s + z, \]  \hfill (11.2)
\[ V(B^N) = w + z + s, \]  \hfill (11.3)
\[ V(h) = w + z + s + t, \]  \hfill (11.4)
\[ V(B) = w + z + t \]  \hfill (11.5)

and

\[ V(A) = V(a) = w + z + t + y. \]  \hfill (11.6)

First, we examine the accessibility of the stable equilibrium states of the system. Because \( V(A) - V(h) = y - s \), the pass at \( h \) is energetically surmountable as \( y \) is clearly greater than \( s \). Likewise \( V(A) - V(H) = y + t - x - s \) is clearly positive, so the pass at \( H \) is also energetically surmountable. All three stable states, \( B, B^N \) and \( B^T \), are therefore energetically accessible.

Second, we compare the energy relaxations achieved. Comparing state \( B \) with state \( B^N \), we have \( V(B) - V(B^N) = t - s \), which shows that they have roughly the same energy levels. Meanwhile comparing \( B \) with \( B^T \), we have \( V(B) - V(B^T) = z + t - x - s \), which, for the sake of argument, we assume to be positive. So, the greatest energy relaxation is achieved if the DNA passes over the two barriers to the accessible straight, trivial, configuration \( B^T \).

12. Discussion

(a) Thermodynamics

In our elastic rod treatment of DNA, the most obvious omission is any detailed consideration of thermodynamics and Brownian agitation. These will clearly play a dominant role, especially at low values of link and writhe, where they are likely to smear out much of the fine detail of the folded equilibrium paths. However, as we have remarked, our classical, deterministic study of the stability of twisted and stretched rods does provide a solid framework against which thermodynamic analyses can be viewed.

Concerning the settling to alternative states, illustrated in figure 9, Strick et al. (2002) describe how strong thermal fluctuations may switch a DNA molecule between a state without plectonemes and a state containing the simplest plectoneme (a single loop). In the absence of Topo 2 they observed that the switching between these two states is so rapid that the extension measured is just an average between the two states. However, adding Topo 2, but without its energy source ATP, the lifetime of the loop is significantly increased by the protein binding (without cutting).
(b) Processivity

We have mainly spoken as if a topoisomerase makes a single cut and then stops. In reality, a topoisomerase will often make not just one cutting cycle, but a rapid succession of cycles in what is called processivity (Strick et al. 2002); such a burst of activity can often remove 30 supercoils at a time. Processivity is easily incorporated into our methodology, though for the simplicity of exposition we have often ignored it.

(c) What happens to the ATP energy?

In §3, we discussed an idealized mechanical operation of the Topo 2, where in elastic rod terms there seemed to be no reason to invoke any energy input, except, of course, to cut the rod. Meanwhile, it is known that Topo 2 uses the energy of ATP hydrolysis to achieve its cutting and rejoining operation (Bates & Maxwell 2005; Maxwell et al. 2005). The following question can then be asked: where, when and how is this energy employed?

The answers to this question are currently being sought by biomedical researchers worldwide. Unfortunately, given that the different enzymes of type 2 do not all have identical mechanisms, it seems clear that no single universal answer will emerge.

The best we can do here is to draw attention to the excellent review by Bates & Maxwell (2007). These authors emphasize that a Topo 2 is an extraordinary molecular machine that utilizes the free energy of ATP hydrolysis to drive energetically unfavourable reactions. In the case of DNA gyrase, the ATP hydrolysis drives the introduction of negative supercoils into the DNA molecule. Meanwhile, in the case of the non-supercoiling type 2 enzymes (topoisomerases II and IV), the principles governing the selectivity of their reactions remain to be established; but the role of ATP hydrolysis is established as the generation of non-equilibrium product distributions.

(d) How multiple cutting can achieve deknotting

In this paper, we have concentrated exclusively on deterministic relaxation (rather than tightening) in a ply of dsDNA. But the much bigger issue involving Topo 2s is about understanding how ‘random’ inter-segmental passages occurring within long molecules in living cells can serve to simplify, rather than complicate, the topological structures involved. For example, how could a local rule guide a topoisomerase (only approx. 5 nm in size), which only senses a neighbouring region of the DNA, to make a cut that will reduce, rather than increase, the global degree of knotting; this is particularly important because knots are known to be ‘toxic’ for cells.

Regarding this, it is clear that however good a local rule is, mistakes will certainly be made by the enzyme. So, what we are looking for is a local cutting rule that will, after a large number of cuts, give a (high) probability of the knotting or twisting levels being decreased. This is illustrated by in vitro experiments where long circular dsDNA molecules (10 kb long) were used as substrates for Topo 2s; the level of steady-state knotting achieved was some 50 times lower than the topological equilibrium that would be generated by a random cutting process, see Rybenkov et al. (1997). Even though
the mechanism may be unknown, this maintenance of a level of knotting below topological equilibrium is thermodynamically possible owing to the ATP energy source.

Recent progress on this problem is reported by Burnier et al. (2007), who have made numerical simulations of a freely jointed chain model to examine how the local geometry of juxtaposed segments of DNA in knotted molecules might guide type 2 enzymes to perform an efficient relaxation of DNA knots. In particular, they studied how the geometrical parameters of inter-segmental juxtapositions at cutting sites can influence the topological simplifications achieved. Their work suggests that by recognizing specific geometric features of the juxtaposed molecule, the topoisomerases can maintain a steady-state knotting level below the topological equilibrium, replicating the findings of the experiments. Finally, they suggest the remarkable result that a preference for a specific local geometry allows the enzyme to select the most efficient pathway of relaxation for a complex DNA knot.

13. Concluding remarks

The link–writhe diagrams of this paper give a useful overview of the various ways in which a cut by a topoisomerase can generate a variety of outcomes, all assigning different levels of relaxation to the molecule. An implicit assumption of the investigation is, of course, that the relaxation after a cut is to another straight plectoneme, rather than to a different form outside the present model. This is certainly a reasonable assumption, but departure from it clearly warrants further investigation, especially as the very thorough and exhaustive calculations of Swigon (1999) uncovered a wide variety of different forms emanating (for example) from higher branching points.

The action of a topoisomerase is strongly governed by the magnitude of the total twist, Tw, measured by the horizontal distance between the equilibrium path and the Lk = Wr line, and the associated area Y of our (Lk, Wr) diagrams.
Now experiments may well be made at high values of the tension parameter, $t = TL^2/4\pi^2 B$, so it is significant that the equilibrium curves of figure 12 start to pull away from this $T_w = 0$ line at high values of $L_k$ and $t$. Note that the non-dimensional tension parameter, $t$, is the physical tension $T$ divided by the magnitude of the Euler buckling load for a compressed rod with clamped ends. The curved asymptote is the result for a uniform unloaded ply (with no tails or end loop) taken from Thompson et al. (2002), eqn (7.9) with $W_r^* = L/(4\pi r)$.

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