Supporting Information

Topoisomerase V relaxes supercoiled DNA by a constrained swiveling mechanism

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Supporting Information

1. Conversion of force to torque for supercoiled DNA

We used an established Monte-Carlo (MC) calculation (1) to determine the torque in duplex DNA as a function of applied force, for a series of values of linking numbers. Calculations were carried out for a 10 kb DNA molecule, using the method and parameters of Vologodskii and Marko (1), which have been shown to quantitatively describe elastic properties of positively supercoiled DNA. Results from the calculations are shown in Figure 5.

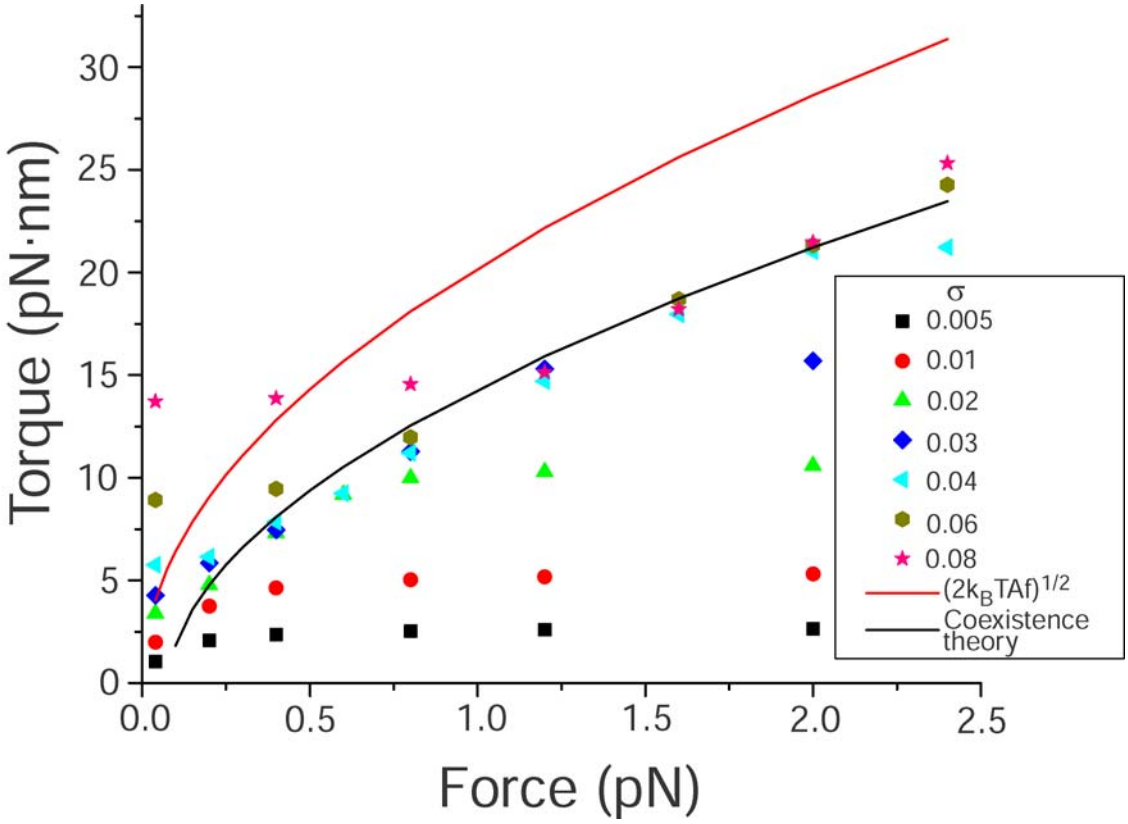

Figure 5. Force *versus* torque plot for 10 kb DNA. Symbols indicate σ = 0.005 (■), 0.010 (●), 0.020 (▲), 0.030 (◆) 0.040 (◀), 0.060 (⬢), and 0.080 (★). In addition, a curve calculated using a formula for the torque of the form $(2k_BTAf)^{1/2}$ (2) is shown in red and a curve calculated using a coexistence theory (3) is shown in black. The coexistence curve is in very good agreement with the values obtained in the MC simulation and was used to translate force to torque.

2. Estimate of viscous drag contributions during linking number relaxation.

Rotational drag on DNA: Consider twist relaxation along the extended part of the DNA molecule. For our 10 kb molecules, the length of molecule relaxing is $L \leq 3$ microns = 3×10^{-6} m in length, and has a hydrodynamic radius $r < 2$ nm. The friction coefficient for rotation of this length of molecule around its axis is:

$$\zeta = 4\pi\eta L r^2$$

Here η is the buffer viscosity; in our experiments $\eta < 3 \times 10^{-3}$ Pa·sec (three times that of water). The rotational drag due to the molecule is therefore $\zeta < 5\times10^{-25}$ J·sec = 5×10^{-4} pN·nm·sec, more than 100 times less than the relaxational friction measured in our topo V relaxation experiments. This is an overestimate since most of the linking number is partitioned into writhe. We conclude that rotation of the DNA molecule around its axis cannot be the origin of the observed friction.

Translational drag on magnetic particle: During relaxation, the viscous drag force on the bead is

$$f = 3\pi\eta\lambda D v$$

where D is the bead diameter of 2.8 microns = 2.8×10^{-6} m, and v is the bead velocity. The dimensionless factor λ accounts for enhancement of the drag due to the presence of the nearby surface (4), and is a decreasing function of extension divided by bead radius. For the 2.8 micron diameter beads used in our study, $\lambda=3$ for a DNA extension of 0.7 microns; λ drops to 2 when DNA extension reaches 1.4 microns. We have only used relaxation events for extensions in excess of 0.7 microns, so we consider the extreme case $\lambda=3$. In glycerol solution ($\eta = 3\times 10^{-3}$ Pa·sec) for velocities in the range we have used (Figure 4A) from 1 micron/sec to 2.4 microns/sec the drag force varies from 0.24 to 0.60 pN. This is well below applied forces from 0.5 to 2.5 pN, indicating that the friction limiting relaxation is not due to the bead in those cases.

For the lowest-force case of 0.2 pN, the drag force on the bead is comparable to the magnetic force in some of our measurements; thus

the torque driving relaxation in the 0.2 pN experiments may be slightly overestimated. However, we have examined the velocities for the groups of 0.2 pN events where the initial extension is above and below 1.0 microns, and we do not find evidence for a strong difference in velocities, as would be expected if the surface were playing a major role in the bead motion. Finally we note that if drag on the bead had been limiting the relaxation, we should have observed a linear dependence of bead linear velocity on applied force. We conclude that drag on the bead is not strongly affecting its motion in most of our measurements.

3. Barrier-crossing model for rotational drag and relaxation step size

In the thermal-fluctuation- and torque- assisted barrier crossing model of Koster *et al.* (5) for the molecular friction associated with topo IB strand rotation, we suppose that following cleavage, relaxation of positive linking number proceeds at a rate

$$r_+ = r_O \ \exp[-\beta E_B + \beta\theta_+\tau]$$

where E_B is the height of the rate-limiting energy barrier, τ is the DNA torque, θ+ is the angular rotation associated with reaching the top of the barrier, and $\beta = 1/(k_BT)$. The factor r_O is the rate of thermal fluctuations, which is approximately $r_O = k_BT/(\eta \ell^3)$, where η is the buffer viscosity, and $\ell \approx 1$ nm is the approximate size of the region of DNA and enzyme involved in fluctuation over the barrier.

In general, and especially for low or negative torques, one must consider reversed rotation as well, which relaxes negative linking number. The rate of reverse rotations is

$$r_- = r_O \ \exp[-\beta E_B - \beta\,\theta_-\tau]$$

The same energy barrier E_B must be crossed, but in the opposite rotation sense. In general the angular rotation to reach the top of the barrier will be different (i.e., the potential need not be symmetric as a function of rotation angle, see sketch in Koster *et al.* (5)), and thus we expect $\theta_+ \neq \theta_-$.

The angular frequency (defined so that relaxation of positive linking number is a positive angular frequency) is the difference between these rates, multiplied by 2π to convert turns per time to radians per time:

$$\omega = 2\pi\left[r_+ - r_-\right] = \frac{2\pi k_B T}{\eta \ell^3} e^{-\beta E_B}\left[e^{\beta\theta_+\tau} - e^{-\beta\theta_-\tau}\right]$$

For small torques the exponentials can be expanded to obtain:

$$\omega = \frac{2\pi k_B T}{\eta \ell^3} e^{-\beta E_B}\left[\left(\theta_+ + \theta_-\right)\beta\tau + \frac{1}{2}\left(\theta_+^{\,2} - \theta_-^{\,2}\right)(\beta\tau)^2 + \cdots\right]$$

For small τ, the relation between ω and τ is linear as we have observed for topo V (Figure 4B); the proportionality constant is the friction constant (see text):

$$\zeta = \frac{\eta \ell^3}{2\pi\left(\theta_+ + \theta_-\right)} e^{\beta E_B}$$

The final exponential factor provides the mechanism for the large amplification of the intrinsic viscous friction (we take $\eta \ell^3$ = 10^{-9} pN.nm.sec) that we have observed. Our estimate of the friction constant of ζ=0.071 ± 0.002 pN.nm.sec determines the energy barrier to be E_B = 19.5±0.2 k_BT or 80.1 ± 0.7 pN.nM or 11.5 ± 0.1 kcal/mol, where the uncertainty comes in part from our measurement errors, and in part from uncertainties in the intrinsic friction and the barrier positions.

For larger τ, the left-right rotation asymmetry in the potential ($\theta+ \neq \theta-$) introduces nonlinear friction which is different for the two directions of rotation. In preliminary experiments we have not been able to detect this difference, and therefore we have fit our topo V data (Figure 4B) assuming the symmetric case $\theta+ = \theta-$, which reduces the number of fit parameters.

We can use our model to understand the size distribution of relaxation events. Following Koster *et al.* (5) we suppose that during rotation there is some angular range δ in which cleavage and ligation occur at rates k and k' respectively. Assuming that the time spent in this angular range is inversely proportional to the rotation rate,

following cleavage, the probability per turn that religation does not occur is:

$$P_1 = \frac{\left(k + k' e^{-(k+k')\delta/\omega} \right)}{(k+k')}$$

Derivation of this formula is discussed below.

Given this, the distribution of relaxation event sizes will follow an exponential distribution with a mean number of turns removed before a religation event of:

$$\langle \Delta Lk \rangle = 1/(1 - P_1)$$

We note that in the limit $\omega=0$ the mean linking number change approaches a nonzero limit

$$\langle \Delta Lk \rangle = 1 + k/k'$$

in accord with our topo V observation of a $\omega=0$ limit of $\langle \Delta Lk \rangle = 11.7$ in 40% glycerol. This indicates that $k'=0.09\ k$, i.e., that the ligation rate is roughly a tenth of the cleavage rate during relaxation. Finally, the change of the average linking number change with angular velocity (Fig. 4c) determines $k\delta$ and $k'\delta$ of 361.4 ± 132.4 sec^{-1} and 33.79 ± 6.7 sec^{-1} respectively.

Our model thus explains the linear and nonlinear friction (see Figure 4B), and the dependence of $\langle \Delta Lk \rangle$ on ω on torque (Figure 4C) that we have observed for topo V. It also predicts a novel breaking of symmetry between relaxations driven by equal magnitude but opposite sign torques. The latter symmetry-breaking effect should occur in general for enzymes which mediate constrained DNA swiveling, e.g., for topo IB.

4. Derivation of P_1

We suppose that following the initial cleavage, during each turn of subsequent relaxation, religation can occur within a reaction zone of angular width δ at rate k'. However, recleavage can also occur at rate k, so that the probabilities of the cleaved (c) and ligated (l) states evolve according to:

$$\frac{dc}{dt} = kl - k'c$$

$$\frac{dl}{dt} = k'c - kl$$

Given initial conditions c(t=0)=1, l(t=0)=0, these equations determine the probability of the cleaved state as a function of time. The probability that the cleaved state will survive through one rotation is P_1 = c(t=δ/ω).

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