Linking number changes in the absolute and apparent sense: Ethidium bromide binding to DNA

The linkage equation is

$Lk = Tw + Wr$

When the DNA is relaxed, these values are:

$Lk0 = Tw0 + Wr0$; $Wr0 = 0$, because in relaxed DNA the axis lies in a plane and there no crossings of the axis.

That is, for relaxed DNA, the linking number is the same as its twist. $Lk0 = Tw0$.

Example: Imagine a covalently closed circular DNA of 1,000 bp. Assume 10 bp turn in the relaxed state. There are 100 turns, so $Lk0 = 100$; $Tw0 = 100$.

If you cut a strand and unwind it 20 times and close it, you have changed the linking number from $Lk0$ to Lk. The DNA is no longer relaxed. Since you remove 20 turns in the DNA, the value of Lk is $= 100 − 20 = 80$. Since your DNA is underwound (less turns than in the relaxed state), the DNA is negatively supercoiled.

The linking number change $\Delta Lk = Lk − Lk0 = 80 − 100 = -20$

The superhelical density σ is change in linking number divided by the relaxed linking number $Lk0$.

$\sigma = [Lk-Lk0]/Lk0 = \Delta Lk/Lk0$

In our example,

The superhelical density is $= -20$ divided by $100 = -0.2$

The ΔLk can be partitioned into both writhe and twist. If it is partitioned entirely into twist, the writhe will be zero but the Tw will change. If the Tw is maintained at its normal value of 10 bp per turn, then ΔLk will be partitioned entirely into writhe. In reality, one gets dynamic transitions into all combinations of Tw and Wr between the extremes of $Wr = 0$ and unit twist $= 10$ bp per turn.

Action of ethidium bromide:

When ethidium bromide binds DNA it unwinds DNA or effectively introduces negative supercoils. Hence, if the DNA is already underwound (negatively supercoiled), ethidium bromide will bind to it with high affinity. Because of the unwinding caused by ethidium bromide, the twist of the DNA is obviously changed. You could think of the bound ethidium bromide as partitioning the writhe in the negatively supercoiled DNA into twist. When the writhe is removed, the DNA looks relaxed, but this is only in the context of the bound ethidium bromide. When enough ethidium bromide is bound to convert all the negative supercoils into twist, the DNA looks fully relaxed, or the apparent $Lk = Lk0$.

If you add still more ethidium bromide, you are causing more unwinding, so the DNA must compensate by overwinding or positively supercoiling.

If you treat the DNA with topoisomerase, the compensatory supercoils are removed. The negative supercoiling present in the DNA is not removed by the enzyme because the bound ethidium bromide protects these supercoils.

You could imagine that topoisomerase changes linking number by processing writhe but not twist. Since the negative supercoiling caused by the binding of ethidium bromide is manifested entirely as change in twist, it is protected against topoiosmerase action.

When the bound ethidium bromide is removed from DNA by dialysis, the negative supercoiling that was confined in the form of twist now appear as writhe, since DNA prefers to maintain 10 bp per turn.

In class today, we illustrated the case of binding ethidium bromide to a relaxed DNA molecule.

In the figure below, I have illustrated the case of binding ethidium bromide to a DNA molecule that contains one negative supercoil ($\Delta Lk = -1$). We added enough ethidium bromide so that the DNA was not only relaxed to an apparent $\Delta Lk = 0$, but introduced a compensatory positive supercoil (apparent $\Delta Lk = +1$). But the real ΔLk of the molecule is still -1, because you have not cut strands and added or removed DNA turns. The molecule contains bound ethidium bromide equivalent to a ΔLk of -2 and a positive supercoil equivalent to a ΔLk of +1. Or the real ΔLk is:

-2 + 1 = -1.

When you add topoisomerase I, it relaxes the DNA in the real senses by changing thie compensatory +1 node to a zero node. So the molecule now contains bound ethidium bromide equivalent to a ΔLk of -2. When you dialyze the ethidium bromide away, you see the real ΔLk of -2 as two negative supercoil (writhe) nodes.

EtBr

$\Delta LK = -1$

$\Delta LK = -1$ (real)
$\Delta LK = 0$ (apparent)

EtBr

EtBr
(dialysis)

topoI

+ve

$\Delta LK = -2$ (real)
$\Delta LK = 0$ (apparent)

$\Delta LK = +1$ (apparent)
$\Delta LK = -1$ (real)

$\Delta LK = -2$