The Energetics and Enzymology of DNA Supercoiling

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by

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To Mom and Dad,

and

Jonathan and Daniel

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Abstract

The dependence of the free energy of supercoiling DNA, ΔG_τ, on the linking number α has been determined for closed circular DNA of 200 to 4400 bp. All the experimental data can be fitted by the relation $\Delta G_\tau = K(\alpha-\alpha^o)^2$; K is a length-dependent proportionality constant, and α^o is a constant for each DNA molecule. For circular DNA larger than 2000 bp, the product of the DNA's length, N, and K is a constant with a value of 700 kcal-bp. For smaller DNA, NK increases steadily as N decreases; for a 200 bp DNA, NK is 2350 kcal-bp. This increase in NK can be explained as the result of a decrease in the writhe of the DNA. The torsional rigidity of DNA can be estimated as 2.9×10^{-19} erg-cm. The large value of K for small DNA allows precise calculation of the helical repeat of DNA. For the 210 bp circular DNA, the repeat is 10.54 bp.

Two studies of the mechanism of action of DNA gyrase have been done. First, the tyrosine in the A subunit of DNA gyrase to which DNA is covalently bound during catalysis has been mapped. The covalent gyrase-DNA complex was isolated following cleavage of the DNA by gyrase in the presence of the gyrase inhibitor oxolinic acid. Two independent methods showed that the site of DNA attachment is tyrosine 122 of GyrA of E. coli.

Second, the action of DNA gyrase on 200 bp circular DNA was investigated. Gyrase inefficiently supercoils small circles, changing the linking number by two. The reaction is likely stoichiometric, not catalytic, yet it requires ATP. Gyrase cleaves small circles efficiently in the presence of oxolinic acid; the specificity of the cleavage is essentially identical to that of linear DNA. The

implications of these results for the mechanism of gyrase action are discussed.

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Chapter 1

An Introduction to DNA Supercoiling

In the canonical structure of DNA the two strands are entwined. This intertwining poses problems for almost every process in which the DNA participates, since the strands must be partially or completely separated. The topology of DNA is complicated by the fact that in many DNA molecules each strand is circular, and the intertwined strands are thus topologically linked. This thesis is concerned first with such circular DNA molecules and their thermodynamics, and, second, with the enzyme DNA gyrase, which can change the topological state of the DNA. This chapter describes some basic DNA topology, as well as the thermodynamics of the various topological states of DNA.

I. The Linking Number, the Twist, and the Writhe

For a double-stranded DNA molecule in which each strand is an unbroken circle (closed circular DNA), the two strands are interlocked: they cannot be separated without breaking one of the strands[1-4]. Different DNA molecules can have different degrees of intertwining of the strands. To quantitate this intertwining, consider the process of breaking one strand of the DNA, passing the other strand through the break once, and resealing the break. The minimum number of such cycles of breaking and rejoining necessary to allow separation of the strands defines the degree of linking of the two strands; this number is called the linking number and is designated α or Lk. Alternatively, the linking number may be calculated by first projecting the image of the DNA onto a plane, and counting the number of crossovers of the two strands[3,4]. This counting procedure yields twice the linking number, since each double-helical turn has two crossovers.

It is clear from the definition of the linking number that it is an integer. By convention its sign is positive for right-handed double-helical DNA[3]. The linking number is topologically invariant; its value does not change under any deformation of the DNA[1,2]. To change the value of the linking number, one of the strands of the DNA must be broken. This invariance, which is not a property of either the twist or the writhe, gives particular importance to the linking number. Molecules with different linking numbers are distinct and isomeric; they are called topoisomers (for topological isomers). Because strand breakage is necessary to interconvert topoisomers, each topoisomer is stable and topoisomers with specific values of α can be studied individually.

The linking number has two components, the twist and the writhe[1-4]. If the linking number of a DNA molecule is calculated by the counting procedure described above, then the crossovers will be of two types: the first will be due to the helical turns of the DNA, and the second, to crossovers of two double-helical DNA segments. These two terms are the twist and the writhe, respectively; they are defined rigorously next. The definitions are essentially those of Fuller[2].

To define the twist (designated Tw), we first define a curve $\underline{X}(t)$ which follows one of the strands of the DNA. ($\underline{X}$ and all underscored letters are three-dimensional vectors.) The second strand follows the curve $\underline{X}(t)+\varepsilon\underline{U}(t)$, where $\underline{U}(t)$ is perpendicular to $\underline{X}(t)$ at every t, and $\varepsilon > 0$. The unit tangent to $\underline{X}$ is called $\underline{T}$; $\underline{U}$ revolves around $\underline{T}$ with angular velocity ω as the DNA is traversed. The twist of the DNA is the integral of ω over the entire length of the DNA: $Tw = (1/2\pi)\int\omega ds$. For each turn of the helix, $\underline{U}$ revolves 2π radians; the integral is