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In the Classroom

Using Rubber Tubing to Demonstrate DNA Supercoiling and the Action of Topoisomerases

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This demonstration could easily be adapted to group learning with small groups of students using the instructions provided here….

I use a simple rubber-tubing model to demonstrate principles of DNA topology, including DNA twist and supercoil, conservation of linking number, and the action of topoisomerases. The resilience of the rubber mimics the natural response of double-stranded DNA to distorting forces such as strand separation during replication. Teachers can use this demonstration in traditional lecture or adapt it as a group-learning exercise.

Introduction

In my biochemistry class, I use ordinary laboratory rubber tubing to demonstrate many of the principles of DNA topology, including the relationship between DNA twist

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and supercoil (also called *writhe*), the conservation of linking number, and the action of type I and type II topoisomerases. These demonstrations derive much of their realism from the natural resilience of rubber tubing. This resilience mimics the natural tendency of DNA to adopt its lowest-energy conformation, the B-form double helix, in the absence of distorting forces.

Most biochemistry textbooks include brief discussions of DNA supercoiling and the action of topoisomerases [1–3]. These sections often arouse student curiosity without providing much understanding, in part because the concepts are difficult to illustrate effectively with static drawings. This demonstration provides a clear picture of all the major concepts required for an understanding of DNA topology. The model I use is inexpensive, so you can make several of them for passing around in a large classroom. This demonstration could easily be adapted to group learning with small groups of students using the instructions provided here to carry out the demonstration and discuss its meaning.

Background

Scovell [4] and Sinden [5] have provided excellent reviews of DNA supercoiling and its biological significance. While the native conformation of B-DNA has one double-helical turn (called one *twist*) per 10.4 base pairs, circular DNA can be isolated in forms that are supercoiled; that is, double strands are coiled around each other. Circular DNA, such as found in some bacteria, poses the problem of how DNA can be unwound for replication. Unwinding—or more precisely, untwisting—at the replication fork forces extra twists into the surrounding regions, twisting DNA out of its lowest-energy (what I will call “natural”) conformation and producing resistance to further untwisting. In circular DNA the double strands respond to excessive twist by supercoiling or writhing around each other, which allows the double strands to move back toward the natural amount of twist.

For a closed circle of double-strand DNA (or for an open double strand restrained from rotation at the ends, such as a region looped out of a packed structure like a nucleosome), the number of twists plus writhes is a constant called *L*, the *linking number*, a term that originates in topology. An example of a system with a linking number of 1 is two links of a simple chain. If you try to lay the two links flat, one of them passes on top of the other one time and behind the other one time. This is one

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link or a linking number of one. If one link is twisted around the other so that it lies on top twice, the linking number is two.

If we represent the number of twists and writhes as *T* and *W* then

$$L = T + W.$$

As long as the DNA strands remain intact, any supercoiling must alter the number of twists, and any change in the amount of twists must alter supercoiling. It turns out that if we define positive twist as right-handed, we must define positive supercoiling as *left*-handed, because left-handed supercoiling conformationally offsets excessive twist (this may not be obvious, but the model will convince you). Thus, introduction of one left-handed supertwist (increase in *W* by 1) should reduce twist (decrease in *T* by 1) and keep the linking number constant.

Molecular biologists have isolated and studied two types of enzymes that function to relieve this conformational strain ahead of the replication fork where the two DNA strands are being untwisted. These enzymes are called *topoisomerases*. Type-I topoisomerases cleave one strand of DNA in a double strand, pass the other strand through the break, and rejoin the ends of the broken strand. This process changes the linking number by one. Type-II topoisomerases draw a length of double helix into a loop, and where the two double strands cross each other, cleave both DNA chains in one double strand, pass the intact crossing double strand through the break, and rejoin the broken chains. This process changes the linking number by two.

I illustrate all these aspects of DNA topology with a circular double strand of rubber tubing. The natural resilience of the rubber mimics the natural tendency of DNA to adopt a low-energy conformation with the result that the model responds realistically to changes in twist or supercoil.

Constructing the Model

Care in constructing the model will assure that it behaves realistically in the demonstration. Cut two four-foot lengths of rubber tubing. It is best if the two tubes are different colors, but, more importantly, they should be the same size and resiliency. (I painted one red tube with a permanent black felt marker.) At one end of each tube insert a plastic tubing connector. Twist the two tubes together so that the red tube

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crosses on top of the black tube eight times. Join the ends using the connectors—red to red, black to black, of course. Let the model dangle from your hand. It will probably twist on itself—this is writhing or supercoiling. Remove the supercoiling by breaking one strand at the connector and rolling one end of the tubing between your fingers while preventing the other free end from rolling. Watch the whole model as you do this, and you will see that rolling in one direction tends to remove supercoils and make the model flatten out. Remove about half of the supercoiling and rejoin the ends; then, break the other tube at the connector and use the same method to remove the rest of the supercoiling. Your goal is a model that hangs comfortably in a flat circle. When you lay it flat, the red tube should cross over the black one eight times. Some fiddling now will pay off later with a model that responds realistically to your manipulations.

By adjusting the model in this way, you are giving it a conformational energy minimum when its strands are twisted around each other eight times, just as DNA possesses minimum conformational energy in its B form, with one twist every 10.4 base pairs.

The Demonstration

Note: You should recommend strongly that students read the pertinent sections of the text before the class in which you present this demonstration. This reading will raise many questions in their minds, and make them much more receptive to the concepts that this demonstration clarifies. In addition, you should practice the demonstration carefully before you try it in class. Be prepared for questions that will force you to examine these concepts in detail.

For the model as constructed, $L = 8$, $T = 8$, $W = 0$. Start the demonstration by defining *twist* (*T*) for this model as the number of times the red tube lies on top of the black tube when the model is laying flat. Ask the class to tell you whether the twist is right- or left-handed (you made it right-handed), and ask them to count *T* (8). Next, show what you mean by *writhe* by twisting the model into a figure eight and then continuing to twist. Define *writhe* (*W*) for this model as the number of times one *double* strand lies on top of the other *double* strand when the model is laying flat. At this point, don't bring up the direction or sign of supercoiling—the model will help you define the signs later.

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State that the sum of *T* and *W* is a constant called the *linking number*, *L*: $L = T + W$. Next state that right-hand twist is signified by positive values of *T*, and ask the class whether right-hand writhing is positive or negative (expect advocates of both answers, especially from those who have not done their reading). Demonstrate the correct answer by beginning to pull the two tubes apart as double strands would separate at a replication fork. As the single strands separate, the double strands on the opposite side of the model spontaneously supercoil around each other. Ask the class the direction of supercoiling (left-handed). Then ask again what is the sign of this supercoiling (positive). Here is the reasoning: pulling the strands apart lowers *T*. The compensating supercoil must be positive, because if *T* is decreasing and *L* is constant, *W* must be increasing. Therefore, positive supercoiling is *left-handed*.

Next, show that *T* and *W* compensate each other precisely. Supercoil the model into a simple figure eight, so that the double strands cross each other just once, in left-handed fashion (make a 180-degree supercoil, don't make a full turn—remember that the definitions of twist and writhe for this model are based on numbers of *crossings*). The left-handed figure eight corresponds to $W = +1$ for this model. Holding the model in this shape, ask the class to count *T*, the number of times that the red tube lies on top of the black one in the double helix. You will find $T = 7$: $L = T + W = 7 + 1 = 8$; therefore, the linking number is unchanged by supercoiling. Now give the model a negative supercoil, $W = -1$, by putting it into a figure eight with a right-hand turn. Again ask the class to count *T*. They will get nine: $L = 9 + (-1) = 8$, as before.

Now, demonstrate dramatically how supercoiling alters twist. Give the model several turns of positive supercoil by twisting it into a figure eight with left-hand turns, and then continue to wind it in the same direction. In the free loops at both ends of the supercoil, you will see that the single strands become almost completely untwisted from each other. The response of the model makes it very plain that the introduction of supercoils alters the number of twists.

Next, demonstrate the action of topoisomerases. Allow the model to relax to $T = 8$, $W = 0$, that is, a flat circle with eight crossings. To illustrate type-I topoisomerase action, break the red tube at its connector, let the black tube pass through the break, and rejoin the red ends. Be careful not to let the ends of the tubes roll with respect to each other. You will have a natural tendency to pass the tube through the break so as to reduce the twist—it's simply easier to do it that way. Now, hold the model flat and ask

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the class to count twists. They should get seven, showing that type-I action changes the linking number by one. If your tubing is very springy, you may be able to see the tendency of the model to supercoil in response to this modification. Let the model hang freely and see if it tends to writhe. My rubber tubing doesn't give much response here, but responds nicely in the next part of the demonstration, on type-II topoisomerase action.

Now, restore the model to its original flat-circle state, $L = 8$, $T = 8$, $W = 0$, by undoing the type-I action (break the red strand, wind one end around the black tube so as to increase the twist, and rejoin the ends). Illustrate the action of type-II topoisomerase as follows. Give the model one negative supercoil as you did earlier (figure eight with the double strands crossing each other once in left-hand fashion), but place the connectors on top at the crossing. Lay the model on a table. Break both tubes at the connectors, being careful not to let the tubing roll in your fingers. Separate the two breaks and (with your third hand or some student help) lift the intact double strand through the break. Rejoin the breaks, taking care (1) not to cross your connections and (2) not to let the tubing roll.

Now, hold the model flat (it resists in a very realistic way, as you will see). Ask the class to count the twists. They should find six. Type-II action has changed the linking number by two, from eight to six. Pick up the model and let it dangle. If you have been careful in this operation, the model will spontaneously adopt some right-hand (negative) supercoil. Now ask the class: with a linking number of six (as shown by counting with the flat model), why should the model supercoil? Here's the answer: the tubing is at its conformational energy minimum when it has eight twists (you made it that way), just as DNA is at its conformational energy minimum when it has 10.4 base pairs per turn. So after you reduce the linking number to six and allow the model to dangle freely, it again seeks the eight-twist conformation, just as DNA seeks the B conformation. To reach eight twists with a linking number of six, the model must supercoil in the negative direction. If the model goes back to eight full right-hand twists ($T = 8$), then it must adopt two right hand supercoils ($W = -2$), so that $L = 6$. In reality (both the reality of the model and the reality of DNA molecules), the conformation is a compromise between the forces of twisting and writhing, so you do not get quite two supercoils.

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Now ask your students for questions. Many students will respond with “what-if” questions about the model or DNA, and seeking answers to them will test *your* understanding of these concepts. Have fun.

How Textbooks Teach Confusion

Terminology in textbooks about the role of topoisomerases can be confusing. For example, it is common to refer to the enzymes as “introducing negative supercoils” to aid in untwisting DNA for replication or transcription. It would be clearer if authors simply stated that the action of these enzymes changes the linking number. If enzyme action *reduces* the linking number, then the twisting of the DNA back toward its natural conformation does indeed result in negative supercoiling, as shown in the demonstration of type-II topoisomerase action. Possessing negative supercoils, DNA strands can separate farther before supercoiling becomes positive and begins to obstruct untwisting. In that respect, it is true that the “introduction of negative supercoils” promotes unwinding of DNA.

Student Reaction

Textbook discussions of DNA topology are not easy reading. I want my biochemistry students not merely to read, but to read critically and refuse to accept anything they read unless they really understand it (or if evidence is presented, unless they see the truth of conclusions in the light of the evidence). Most of them cannot read critically any existing textbook discussion of DNA topology. I have yet to hear a single student say that he or she understood these concepts clearly before seeing some kind of model. One bright student this year told me that he simply had to accept the statements in the text without question and could not imagine a way to verify them. “Now,” he said, “I understand.”

The most encouraging part of student reaction to this demonstration has been the quality of questions during and after the demonstration. It is clear to me that students are probing these subjects more deeply and with greater insight than they did before I began using this demonstration.

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