Electron Paramagnetic Resonance: A New Way to Determine the Structure and Function of DNA

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Background

Kenneth Tham is a junior at the University of Southern California, majoring in Chemical Biology and minoring in East Asian Languages and Cultures. As a freshman, Kenneth's interest in research sparked when he enrolled in Dr. Takahashi's Chem 115b course. Dr. Takahashi focused on research that involved the use of lasers to look at biomolecules. After speaking with Dr. Takahashi and showing his interest in the field, Kenneth was referred up to Dr. Qin's lab, which is where he has been working ever since.

The Basics: What exactly is a spin label and what does it do?

Before we talk about spin labels, it is important to understand the interaction between molecules and magnets. Generally, when a molecule is exposed to a magnetic field, the

molecule spins because each molecule has some sort of ionic charge, which reacts with the magnet. However, when DNA is placed into a magnetic field, it does not spin unless a spin label is attached to it. Spin labels are stable molecules, despite having two unpaired electrons. Once a spin label is placed into a magnetic field, the magnet either amplifies the spin or reduces the magnetic field.

The instrument used that detects the unpaired electrons on the spin label and cause it to spin is called electron paramagnetic resonance (EPR). How and where the spin label is attached to the DNA molecule determines how the molecule will move.
Dr. Qin's lab focuses on how Site Directed Spin Labeling (SDSL), the interaction between the DNA, spin label, and magnetic field, can evolve the structure of DNA. Usually, spin labels are attached to proteins; however, Dr. Qin's lab is unique in that it is one of the only labs that centers in on the interaction between spin labels and DNA.

How do the spin labels attach to the DNA molecule?

A DNA molecule is made up of three components: a deoxyribose sugar, a phosphate backbone, and nitrogenous bases. The spin labels attach to the phosphate molecules in the DNA structure. The structure of phosphate and how it attaches to the deoxyribose sugar in DNA can be seen in Figure 1.

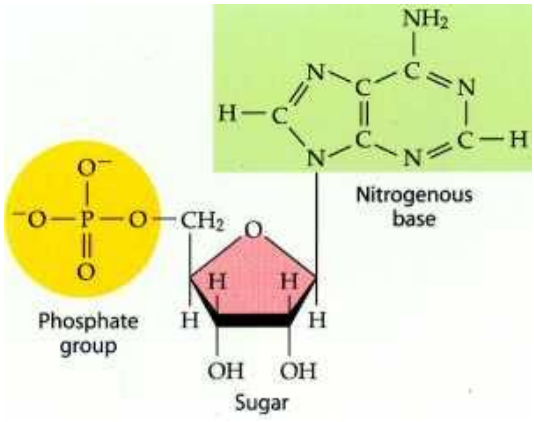

Figure 1: The structure of a phosphate molecule is circled in yellow. It consists of a phosphate atom (P) and four oxygen atoms (O). The phosphate group attaches to the deoxyribose sugar in DNA. http://www.dnareplication.info/images/dnadoublehelix.jpg

The spin label, however, cannot attach directly to an oxygen atom on the phosphate group because the interaction is not very reactive. Dr. Qin developed a method called Phosphorothioro labeling scheme, which removes the outermost oxygen atom on the phosphate group of DNA and substitutes it with a sulfur atom which reacts more directly with the spin label than oxygen (Figure 2).

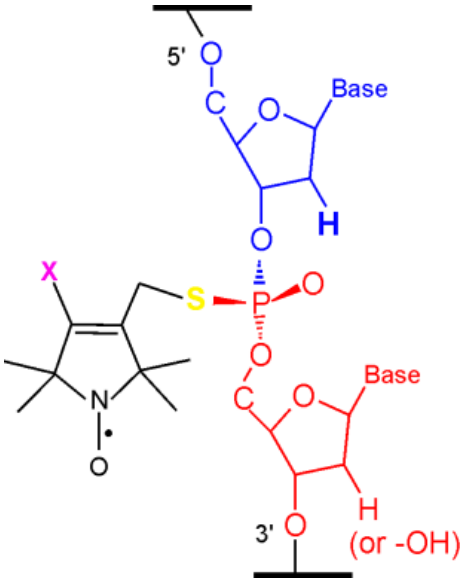

Figure 2: Here is an image that shows the interaction between the phosphate group and the spin label. Phosphate group is between the two deoxyribose sugars. The spin label is the molecule that is black. Notice that the outermost oxygen atom is replaced with a sulfur atom, allowing the phosphate group and spin label to interact directly.

Exploring the Kinetics

Kenneth's research within Dr.Qin's lab deals with the kinetics of the interactions between the spin label and DNA. Kinetics explores the movements of molecules and the physics associated with those interactions. He determined that the labeling reaction between the spin label and DNA is a lengthy process, requiring approximately 24 hours. Kenneth is attempting to streamline this process to make it more effective by using rate laws to deduce the reaction rate of the DNA and spin label junction. Rate laws are sets of equations that calculate the rate of reaction of a specific chemical equation. The basic equation for a rate law is:

$$\text{Reaction rate}=k[A]^n[B]^m$$

where k is the rate constant, [A] and [B] are the concentrations of the molecules, and n and m establish the order of [A] and [B]. The rate law for the interaction between the spin label and DNA is: $r=k[\text{DNA}]^n[\text{spin label}]^m$. Since the order for both DNA and the spin label are one, the overall order for the reaction is two (Figure 3).

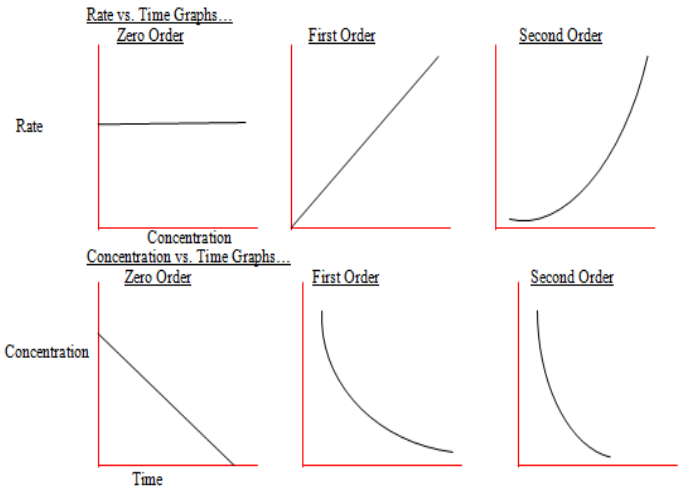

Figure 3: These are the graphs for zero, first, and second order reactions. The first line shows Rate vs. Time graphs whereas the second line shows Concentration vs. Time graphs. http://2.bp.blogspot.com/-5D0RHPSgyz0/TZpioN6QpTI/AAAAAAAAAAY/36MmzOD7_Ug/s640/Concentration+v+Time+Graphs.png

Through his research, Kenneth was able to resolve that the order for both [DNA] and [spin label] is one. The spin label that attaches to the DNA phosphate group is known as the R5 spin label. However, in order to establish the concentration of the R5 spin label, another spin label must enter into the equation. The steps are as follows: first, a spin label known as HO346 is reacted with Sodium Iodide (NaI). Next, the Sodium Iodide (NaI) transforms the HO346 spin label to the R5 spin label that attaches to the DNA. Kenneth discovered that the reaction between the HO346 spin label and the Sodium Iodide (NaI) goes to completion, meaning that there is nothing left over from the reaction. He also found out that the R5 spin label does not degrade after 24 hours and is still reactable.

Kenneth is currently attempting to solve the last piece of the rate law puzzle, the true rate constant k for this reaction. Once Kenneth calculates the true rate constant, he will be able to dictate what concentrations of DNA and the R5 spin label will result in a faster reaction rate.

What is the significance of spin labeling?

Currently, there are two methods to evolve the shape of DNA. The first method is called X-Ray crystallography, which requires making a perfect crystal and shooting X-rays at it to determine the shape of DNA. The disadvantage of using this method is that it is extremely difficult, time consuming, and expensive to make a perfect crystal. The second method, called Nuclear Magnetic Resonance (NMR), uses radiowaves to excite the spin movements of particles in the nucleus or electrons in the shell. These spin movements allow for the evolution of the shape of DNA.

Dr. Qin's lab wants to make SDSL usable so that X-ray crystallography and NMR are not the only methods to resolve the shape of DNA. Also, the structure of DNA is based on how proteins bind to it. So certain proteins will not be able to bind to DNA based on the shape of the DNA molecule. Using SDSL to resolve the shape of DNA, it can be determined which proteins can and cannot bind to the DNA molecule. The presence of a protein or lack thereof could potentially lead to a disease. In essence, SDSL can have a huge impact on medicine because it will make it easier to determine which diseases a person has. SDSL also can have a huge impact on the pharmaceutical industry because it can help people create medicine that can directly affect the area where the disease originates.

Despite being the only undergraduate student in Dr. Qin's lab, Kenneth is given a lot of freedom to run his own experiments and determine his own results. If all goes well and he is able to determine the true rate constant for the rate law equation, he will begin work on a scientific paper in which he will be the primary author.

After his undergraduate years at USC, Kenneth hopes to attend Pharmacy school. His research directly correlates to his career of choice because SDSL will streamline the creation of pharmaceuticals. However, Kenneth's ultimate goal is to become a teacher for high school or college students.