A Single Molecule Force Spectroscopy Study of the Insulin-G-Quadruplex Interaction

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This summer I studied the interaction between the protein, insulin, and a specific region of DNA. The region of DNA that we looked at is called the Insulin-Linked Polymorphic Region (ILPR) and it is located near the promoter for the insulin gene. Promoters are where transcription factors can bind and start the transcription of a specific gene. There are three different alleles, or classes, of this region and the shortest class is associated with type one diabetes. One of sequences found in this region has been found to be able to bind with insulin. This may mean that insulin regulates its own transcription. We wanted to study this interaction to learn about the binding properties of insulin and the DNA which, in the future, may have an implication in diabetes.

The sequence that we looked at this summer forms non-traditional structures of DNA called G-quadruplexes. When there are repeating guanine nucleotides in a section of DNA, they can form hydrogen bonds with each other and make a layer called a G-quartet. Multiple G-quartets can stack on top of each other and form a G-quadruplex.

We studied the interaction of insulin and DNA using the Atomic Force Microscope (AFM). This is not like an optical microscope that one looks through and sees a magnified image. Instead, this is a microscope that measures mechanical properties. The microscope has a cantilever with a very fine tip which can probe a flat surface. If a ligand is attached to the tip, and a receptor to the surface, the force of the interaction can be measured. We used the microscope to measure the force of the interaction between insulin and G-quadruplex DNA. This was done in two different methods. In the first approach, insulin was immobilized on the surface and the G-quadruplex was attached to the tip. As the tip and surface approached, the two could interact and the force of that interaction could be measured as the tip and surface retracted and the binding was ruptured. The disadvantage of this method is that the insulin takes a random orientation on the surface which limits the binding to the DNA. The second approach had the G-quadruplex on the surface and free insulin in the buffer, therefore we don’t have to worry about the orientation of the insulin.

When using the AFM and applying analysis models to the data, we can extract kinetic information such as the $k_{off}$ which is the intrinsic dissociation rate of the interaction, the barrier width which is the distance from the completely folded state to the transition state, and the free energy of activation. After applying the models to our approaches we found that approach one produced much cleaner data than approach two. The interaction of the insulin and DNA lasted for about 0.2 seconds, and the energy of activation is where expected for a non-covalent interaction. The results are important because there is no other technique available to find kinetic information at the single molecule level.

Doing research this summer was a good experience. It was a very different summer than last as last summer was spent running experiments and this summer was spent doing analysis and getting data ready for a publication. Last summer I learned how to work with a group of people on an experiment, and this summer was spent mostly analyzing and coming to conclusions alone. I learned that there is a LOT of work that goes in to getting data ready for publications. I am very grateful for this experience to work on this project for a second summer and learn new aspects to the research process.