Adam Hilbrands
Professor David Benson
Summer Research 2016

Mechanistic Study of Crosslink Formation in BF4112

Posttranslational modifications occur in proteins and have many important roles in cellular activity and biochemistry. Crosslinked modifications are particularly important in metabolism. However, these crosslinks have only been identified by crystallography, which is a difficult process. Because of this, few crosslinked proteins have been identified. We have developed a way to identify crosslinks using fluorescence, which is much easier than using crystallography.

The crosslink we are studying occurs in the protein BF4112, which comes from *bacteroides fragilus*; a gut bacteria. We have demonstrated that a crosslink forms between cysteine and tyrosine via copper. I have been working on proving that the crosslink forms between the specific cysteine and tyrosine in BF4112 that we think it does, and that copper is necessary for the crosslink formation.

To do this there were three main steps I followed. First, I had to dialyze copper into the BF4112. Then I had to reduce the BF4112 and expose to oxygen. Lastly I measured it with absorbance and fluorescence to quantify how much crosslink had formed. To do the dialysis the BF4112 solution would be put in a dialysis bag which would be placed in a copper sulfate solution. This would get the necessary copper into the protein. When that is done, sodium dithionite would be added to the solution to reduce the copper. This has to be done in an anaerobic bag so that no oxygen got into the solution. When there was enough sodium dithionite in the solution it would be taken out of the anaerobic bag and exposed to oxygen, which would oxidize the copper and form the cysteine tyrosine crosslink. Fluorescence measurements would then be taken of the BF4112 at pH 10 and pH7 so that I could quantify how much crosslink had formed.

My results have shown that the tyrosine that we thought was forming the crosslink is indeed the correct one, and that copper in necessary to form the crosslink. When I tried doing the experiment with a form of BF4112 that lacked the necessary tyrosine no crosslink formed. I also tried doing the experiment and dialyzing zinc into the protein instead of copper and again no formed. By comparing my results to a student who worked before me, I also know that one hundred percent of the BF4112 formed the crosslink.

My summer research experience has been incredibly beneficial to me. After Calvin I hope to go to grad school to study chemistry and get a Ph.D. Spending a summer doing research has been very helpful to me so that I can get a taste of what doing research in grad school will be like. I also learned a lot; about things I learned in class and about lab techniques. Doing research has helped me gain a better understanding of things I have learned by giving me firsthand experience working with them. I also gained incredible experience in lab work. There are many instruments that I had never used before but now feel comfortable operating. I also learned many lab techniques to make things go better or faster that will be invaluable to me. I have loved my time doing research at Calvin for Professor Benson and look forward to doing more research in grad school.