SOAR Research – Faculty Proposal – Summer, 2014

DNA Unwinding by Cytotoxic Rhodium Compounds

Faculty: Shari U. Dunham, Ph.D. and Stephen Dunham, Ph.D., Associate Professors of Chemistry

Student: Kathleen Sicinski
Project Start Date: May 27, 2014
Length of Project: 10 weeks

Description of the project
DNA Unwinding by Cytotoxic Rhodium Compounds

Some transition metal complexes, when they enter a living organism, can cause cells in that organism to undergo programmed cell death. One such metal complex is commonly known as cisplatin, contains a platinum atom at its core, and is believed to cause cell death by binding to a cell's DNA and interfering with normal DNA processing. Since cisplatin is an effective drug for the treatment of a variety of cancerous tumors, we make and study other transition metal compounds that bind to DNA, interfere with normal DNA processing, and lead to cell death (are cytotoxic or "toxic to cells") in the hopes of identifying potential new antitumor drugs.

Our research has shown that, like cisplatin, a series of rhodium (Rh) compounds can bind to double-stranded DNA and that minor changes in the functional groups of these rhodium compounds can greatly affect the rate at which they bind to DNA.[1,2] Most recently, we have identified that certain functional group changes in these compounds also affect the kind of DNA adducts they form (intrastrand vs interstrand). We would now like to explore if DNA conformation changes may also be affected by the functional groups in the rhodium compounds. Our goal this summer is to use large amounts of a readily available DNA plasmid to measure one such DNA conformation change, the unwinding angle, which can result from the DNA-binding of these promising rhodium compounds.

Former honors student Alexander Servin ('13) worked throughout the '12-'13 academic year to take a known method of measuring DNA unwinding by metal compounds and adapt it to the resources we have here at Moravian College.[3] By April of 2013, Alex clearly showed that we could successfully generate and purify the quantities of plasmid DNA required for these studies,

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[1] S.U. Dunham, A.E. Burr, S. Mikulski, H.T. Chifotides and K.R. Dunbar, "Covalent Binding and Interstrand Cross-Linking of Duplex DNA by Dirhodium(II,II) Carboxylate Compounds", *Biochemistry* 44(3), pp 996-1003 (2005).

[2] S.U. Dunham, T.S. Remaley, B.S. Moore, D.L. Evans and S.U. Dunham, "Isolation, Characterization, and DNA Binding Kinetics of Three Dirhodium(II,II) Carboxyamidate Complexes: $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ where L= $[OOCCH_3]^-$, $[OOCCF_3]^-$, $[HNOCCF_3]^-$", *Inorganic Chemistry*, **50**, pp 3458-3463 (2011).

[3] Servin, A. "DNA unwinding by antitumor active dirhodium compounds" (May 2013) Honors Thesis, Moravian College, Bethlehem, PA 18018.

modify plasmid DNA with incremental known amounts of metal compound, and systematically determine the DNA unwinding angle for a control metal compound, cisplatin. Ghazal Stity ('15) has most recently continued this work in our laboratory during the winter term (Jan '14) and now into the spring semester of 2014. Ghazal has isolated large amounts of plasmid DNA and is currently optimizing experiments to determine the DNA unwinding angle of a commercially available dirhodium compound from our early studies.[1] The main goal of this summer project is to use the same protocol to measure and compare DNA unwinding angles for several new dirhodium compounds that we have recently synthesized and begun to study in our research laboratory.

Roles and Responsibilities

- A project director (Shari or Stephen Dunham) will be available to train Katie on DNA isolation and purification procedures, instrumentation, Rh-DNA reactions, gel analysis of DNA reactions, data analysis in excel, and visual representation of gel results in Microsoft Word or Powerpoint.
- Katie will prepare and present at daily meetings (~15-30 min) with project directors (Shari and/or Stephen Dunham)
- Katie will maintain a research laboratory notebook that will include regular and complete entries. The laboratory notebook will have an updated table of contents at the beginning. Entries should be dated, clearly written and organized, and made at least daily with details of ideas for experiments, planning of experiments, clear reference to location and organization of electronic data for each experiment, and a summary of results from each experiment. Project directors will look at Katie's notebook periodically and provide informal feedback throughout the summer. The notebook will be submitted to the project directors upon completion of the Summer Research.
- Throughout the summer, Katie will prepare a summary figure for each set of experiments (with detailed figure caption!) to clearly illustrate the results of each experiment. These summary figures will be submitted electronically to the project directors before completion of the Summer Research.
- Katie may consider continuing this research in an independent study during the 2014-2015 academic year, so a final report/poster may not be required at the end of the summer but instead by the end of the independent study experience and in time for the Annual Student Scholarship and Creative Endeavors Day in spring of 2015 .

Project Timetable

- ☐ Week 1: Safety training, lab orientation, pipettor calibration check, measuring DNA concentrations by UV-Vis, measuring Rh concentrations by GFAAS, checking puC19 by agarose gel (preparing, running, staining, imaging, preparing a gel results Figure)
- ☐ Weeks 2-4: Prepare large quantities of pUC19 plasmid DNA either from pUC19-containing XL1-blue cells (generated by A. Servin in Feb 2013) or by preparing a new batch of XL1-blue cells to contain pUC19 plasmid (sterile lab technique, checking quality and concentration of isolated plasmid stocks)
- ☐ Weeks 5-7: Plan and prepare a series of reactions between plasmid DNA and a novel rhodium compound from our more recent work[2] to determine a DNA unwinding angle (DNA-precipitation, determination of amount of rhodium bound to DNA in each

reaction, identify coalescence point from agarose gel analysis, calculation of unwinding angle, reproduce results at least 2 times)

- ☐ Weeks 8-9: Repeat DNA-unwinding reactions and measurements using at least one additional novel dirhodium compound.
- ☐ Week 10: Perform any replicate measurements, prepare final gel figures for presentation, complete lab clean up and appropriate cataloging of samples and reactions.

Summary of benefits

Student engagement in discipline-appropriate scholarly research. Katie will be engaged in biochemistry laboratory research that includes reading and summarizing primary literature, planning and performing experiments that require the use of sterile lab techniques and several new instrumental methods, and collecting/analyzing/organizing significant amounts of electronic data. In addition she will prepare and receive feedback on various visual representations of experimental results (preparing figures in a format appropriate for publication in a Chemistry journal). The Drs. Dunham will work with Katie to prepare a scientific poster for presentation of this work at a local and possibly a national conference.

Impact on faculty, campus community, and discipline. Katie's work on this project will contribute to our knowledge relating the structure of dirhodium compounds to their ability to unwind the DNA double-helix. Our intent is to combine her data with our other results on the DNA-binding rates and DNA-binding modes of a series of novel dirhodium compounds and publish on how these may relate to their ability to enter and kill various cancer cell lines (work begun on our Spring 2013 sabbatical in collaboration with Dr. Damien Thevenin in the Department of Chemistry at Lehigh University). Her proposed summer project will also benefit the Dunhams by building upon the work of two former research students and possibly continuing the research during the 2014-2015 academic year. Katie will be encouraged to present her work at local and national meetings and she will be required to present her results during the Annual Student Scholarship and Creative Endeavors Day in spring of 2015.

Proposed Expenses (beyond stipends)

$200 Competent cells with heat-shock transformation kit (contains: 20 x 50 microL XL1 blue *E. Coli* cells, 50 pg/microL pUC19 plasmid, 1x SOC media); Alex Servin found this cell strain to work well for production and isolation of many micrograms of pUC19 plasmid. This kit would give us enough reagents to perform this transformation more than a dozen times if necessary and allow us to isolate the milligram quantities of the plasmid that will be required for the proposed unwinding experiments.

$200 Graphite tubes (pk of 5) for PE AAnalyst 700 spectrometer. These tubes are consumable parts of the spectrometer that need to be replaced every 100-200 measurements and are required to determine the amount of rhodium that is bound to a pUC19 DNA sample in an unwinding experiment; we will likely use 2 or 3 of these tubes during a summer of research.

$100 Plastic petri dishes, LB media mix, agar, sterile loops, sterile filters. These are all consumable lab materials required for cell cultures that we have borrowed from Biology in the past but would like to be able to replace/replenish stocks as we use them.

$500 Total laboratory expense request

Kathleen Sicinski
Biochemistry Major
Moravian College Class of 2015
Project Title: DNA Unwinding by Cytotoxic Rhodium Compounds
Mentors: Dr. Shari Dunham and Dr. Stephen Dunham
Requesting On-Campus Housing

Since I started my undergraduate education, my major focus has always been in the sciences. Chemistry and biology have been chief influences in my life. Not only are they concepts that are involved in ordinary life, but my family members are all focused on professional scientific fields. My aunt, Dr. Mary Kay Francis, was a molecular biologist and professor at Villanova University. Before Villanova University, Dr. Francis researched cures for certain cancers at Fox Chase Cancer Center in Philadelphia. Unfortunately, I was not able to ask her the many questions I have about her research. At the age of 50 years, Dr. Mark Kay Francis passed away.

This death had a profound influence on my younger self. Being only 12 years old, I promised myself that I wanted to be like her and research cures for cancer. When I transferred to Moravian College in the spring of 2013, I began to see a way to fulfill this promise. Participating in the SOAR project with Drs. Shari and Stephen Dunham, would fulfill my aspiration. They have explained to me the "big picture" of their research and for this project they plan to use various rhodium complexes to cross link DNA. This cross linkage results in the body terminating that specific cell. This tagging can then be applied to cancerous cells. Although this project does not directly research a cure for cancer, all of the concepts and techniques I learn could be applied to identification and elimination of cancer cells. It is fascinating how alterations in DNA can result in the termination of a cell's life. I am excited to be involved in this project and develop new skills to apply in my professional life.

The research knowledge and skills gained from of this summer project would be invaluable to me. This opportunity would be my first experience in a laboratory setting outside of a traditional semester-long course. I will be working independently with the guide of professors, primary resources such as published scientific papers, and other students' laboratory notebooks. The involvement in Moravian's Summer Opportunities of Academic Research program will formulate the next level of learning for me as a scientist. Although I am in the planned path of medical school, scientific research is still on my mind. This opportunity will give me a strong foundation in the sciences and self-confidence in whichever field I choose. The specialization in the laboratory over the summer, would build on all the knowledge developed in the classroom. This type of "hands on" education is significant and not available to all undergraduate students before they leave Moravian and enter their chosen career path. This preparation is unparalleled, incomparable, and invaluable to my professional development.