

Proposal for Senior Honors Thesis

HONS 497 Senior Honors Thesis

Thesis Title: Lectin Properties of Synthetically Produced Glucuronate, Alginate, and Related
Boronates

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Description of Research

Introduction

Lectins are proteinaceous macromolecules of nonimmune origin, capable of interacting with carbohydrates to form complexes (Goldstein, 1980). Lectins are derived from a variety of sources. They are widely seen in seeds of leguminous plants, animals, fruiting bodies of fungi, and bacteria.

In the 19th century, researchers discovered the ability of some proteins to agglutinate red blood cells (Goldstein, 1980). These proteins, mainly found in the seeds of leguminous plants, were named *phytohemagglutinins*, or *hemagglutinins* (Gorakshakar, & Ghosh, 2016). Later, particular hemagglutinins were found to agglutinate red blood cells (RBC's) of a particular human blood type in the ABO blood group system. As a result, in 1954, Boyd and Shapleigh named these hemagglutinins “lectins” from the Latin word *legere*, meaning “to choose” or “to select” (Goldstein, 1980).

In 1888, Peter Hermann Stillmark isolated toxic extracts from seeds of the castor tree (*Ricinus communis*), and found that these hemagglutinating proteins agglutinated erythrocytes and named them “ricin” (Lis & Sharon, 2004). Similarly in 1891, as the 19th century sparked collaborative efforts for early research in protein agglutinating activity, H. Hellin isolated *abrin*, toxic protein obtained from the seeds of jequirity bean (*Abrus precatorius*), which is similar in structure and properties to ricin (Dickers, Bradberry, Rice, Griffiths, & Vale, 2003). However, it was not until 1919 that Sumner isolated extracts from jack bean seeds (*Canavalia ensiformis*), purified it for the first time, and named it concanavalin A (Goldstein, 1980).

Further research into the agglutinating properties of lectins quickly ensued with a particular focus on research aimed at identifying new lectin sources. In 1940, William Boyd and

Karl Renkonen discovered that extracts of lima bean, *Phaseolus limensis* agglutinated blood type A, but not type B or type O (Lis & Sharon, 2004). In 1959, G. W. G. Bird reported that precipitins from *Dolichos biflorus* seeds reacted with part of the A-substance of human red blood cells; specifically, an A-substance component found in individuals of sub-groups A₁ and A₂ (Bird, 1959; Etzler & Kabat, 1970). Extracts from the asparagus pea, agglutinated type O human red cells but no other types (Sharon & Lis, 2004). Lectins continue to be the focus of research due to their potential and diverse applications specifically for blood grouping, mitogenic activity, challenges related to polyagglutination, and stem cell transplantation (Hammid, Masood, & Rafiq, 2013). There has been great effort in characterizing legume-derived lectins (Hamid, Masood, Wani, & Rafiq, 2013; Lagarda-Diaz, Guzman-Partida, Vazquez-Moreno, 2017). However, the study of lectins remains a prolific field with novel lectins becoming the focus of recent studies (e Lacerda et al., 2017; Torres et al, 2019).

Specific applications of lectins hinges on meticulous characterization of their properties and the identification of carbohydrate-specific binding sites. This research will focus on determining lectin properties of synthetically produced Glucuronate, Alginate, and related Boronates. These compounds have been previously screened for Lectin properties; however, specificity was not in the scope of the study (Koshar, 2018). In addition to confirming previously described lectin properties for Glucuronate, Alginate, and related Boronates, this research aims to categorizing the compounds according to their lectin-characteristic structures followed by their selective interaction with human red cells.

Methodology

The agglutination of human red blood cells will be used as an indication of lectin activity. Agglutination is a standard serological method in the clinical laboratory to detect antibody-antigen interactions through visible clumping or agglutination.

In immunohematology, commonly known as blood banking, determinations of antigen and antibodies are the key to identifying blood groups, unexpected antibodies, and making sure blood compatibility is achieved. Antibodies are glycoproteins capable of recognizing a specific antigen. Antibodies concerning the blood bank are present in the serum and plasma. In contrast, antigens relevant to immunohematology are found on the cell membrane of red blood cells. Hemagglutination occurs when an antigen binds with a corresponding antibody. In the blood bank, hemagglutination is graded on basis of 4+, 3+, 2+, 1+, Negative. A 4+ is a visible red cell solid agglutinate with a clear background. A 3+ are several large red agglutinates with a clear background. A 2+ are may medium-sized agglutinins with a clear background. A 1+ are many medium and small sized agglutinates with a reddish turbish background. Negative, the absence of agglutination.

While previous research indicates that red cell agglutination has been observed with certain synthetically derived compounds, studies on specificity were not conducted (Koshar, 2018). Therefore, the focus of this procedure will be to first confirm previous findings reported on initial testing followed by specificity testing. The proposed testing protocol is describe below.

Type A, B, and O human red blood cells, from donated units will be used. Donated blood is collected bags containing acid citrate dextrose (ACD)-A, which contains 3% citrate to prevent coagulation and maximize cell life. A 2-5% red cell suspension in saline will be prepared as follows: To a labeled tube add 1 mL of whole blood. Wash cells in saline for 5 minutes four

times and removing last aspiration and keeping the pellet. Add .3 mL of washed cells to another tube with 9.7 mL of saline. Cap tube with parafilm and shake to obtain 2-3% red cell suspension or centrifuge for 15 seconds a total of 3-4 times and adding saline to achieve the correct red cell suspension.

A stock solution for each compound will be prepared by adding 0.02 grams of each of these products will be taken and combined with 100 mL of DMSO. Mix until dissolved.

Testing for hemagglutination will be performed as follows:

1. Label test tubes with respective compounds.
2. Add one drop of compound (ID) stock solution to each test tube.
3. Add 2 drops of 2-5% human red blood cell suspensions (refer to procedure for preparing a 2-5% red cell suspension)
4. Centrifuge for 15 seconds.
5. Examine tubes both macroscopically and microscopically for the presence of agglutination.

Results will be described and evaluated. The lectin characteristics of each compound will be thoroughly discussed.

Project Originality

This project is unique because it combines my knowledge of blood banking with ABO groups, type and screen, antibody-antigen interactions and applies it to synthetically derived compounds. Additionally, this project will add to the knowledge base of lectins by describing the characteristics of potential novel lectins from synthetically derived compounds. This research also expands on previous research done with the synthetic compounds by adding the immunohematology perspective. The laboratories in the Department of Medical Laboratory Sciences provide the necessary equipment and supplies for me to complete this project.

Annotated Bibliography

Dickers, K. J., Bradberry, S. M., Rice, P., Griffiths, G. D., & Vale, J. A. (2003). Abrin poisoning. *Toxicology Review*, 22 (3), 137-142.

This review allowed me to further analyze the properties of abrin, a lectin from the seeds extracts of the jequirity bean. Additionally, I was able to further analyze how this lectin works in terms of analyzing a lectin from a bean. Some of the effects of consuming jequirity is gastrointestinal activity. This reference helped me in analyzing the characteristics of abrin, an early lectin, in details and their effects in human health.

Fung, M. K., Eder, A., Spitalnik, S. L., & Westhoff, C. M. (2017). *Technical Manual*. Bethesda, MD: AABB.

The technical manual is the most imperative guide in the field of bloodbanking. It has often been called the “bible of blood banking” because it includes the procedures and methods for routine testing in immunohematology as well as gives guides as to what each

test means in terms of diagnosis in the laboratory. I used this technical manual to get the procedure about agglutination testing with the tube method, grading of tube method, and the process for obtaining a 3-5% red cell suspension.

Ghazarian, H., Idoni, B., & Oppenheimer, S. B. (2011). A glycobiology review: carbohydrates, lectins, and implications in cancer therapeutics. *NCBIS*. doi: 10.1016/j.cthis.2010.02.004

This Review was helpful in giving me first a better understanding of lectins and their characteristics as a whole with being carbohydrate binding proteins, rather than discussing the history of lectins. Additionally, this review talked about the implication of lectins in cancer research.

Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T., & Sharon, N. (1980). What should be called a lectin? *Nature*, 285(5760), 66–66. doi: 10.1038/285066b0

This reference was important in understanding the final term that described what lectins consisted of. The early lectins that were discovered, primarily, ricin and abrin were found to agglutinate red cells. Later on, other lectins were found to agglutinate particular red cells. This source was able to summarize the definition of a lectin taking into consideration the sugar binding capability. I think it was important to understand the final definition of a lectin in order to understand the role lectins serve in immunohematology in the broad spectrum.

Gorakshakar, A., & Ghosh, K. (2016). Use of lectins in immunohematology. *Asian Journal of Transfusion Science*, 10(1), 12. doi: 10.4103/0973-6247.172180

This article talked about the implications lectins play in the biomedical world, including being applied for therapeutic drugs as well as having antitumor, antimicrobial and antiviral effects. I used this review in order to have a better understanding as a potential for further research that lectins can play after being discovered from different sources.

Howard, P. R., & Blaney, K. D. (2017). *Basic & applied concepts of blood banking and transfusion practices*. St. Louis, MO: Elsevier.

This reference enhanced the technical manual in terms of describing the rationale between agglutination and specificity. This source also mentioned how *Dolichous biflorus* is used in blood banking to determine A1 subgroups.

Kosher, E. (2018). Synthesis and Agglutinating-Coagulating Properties of Glucuronate, Alginate, and Related Boronates

I used this source to see the compounds that were able to agglutinate based on synthetically derived compounds. The compounds in my project are donated from the department of chemistry. I further test these compounds for lectin specificity and characteristics that was not previously done.

Lis, H & Sharon, N. (2004). History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*, 14(11). doi: 10.1093/glycob/cwh122

This article talked about the history of lectins from Peter Stillmark in 1888 who isolated toxic extracts from the seeds of the castor tree and named the extract, ricin. Stillmark later was able to say that the ricin, highly toxic, agglutinated red blood cells. The 19th century marked collaborative efforts in the research of lectins including Stillmark with ricin, “abrin” in 1891 by Hellin from the jequirity bean. In 1900, Karl Landsteiner was able to talk about the ABO specificity that lectins played in blood banking and said that the extracts resembled antibodies in all aspects when they agglutinated red blood cells.

Hammid, R., Masood, A., & Rafiq, S. (2013). Lectins: Proteins with Diverse Applications. *Journal of Applied Pharmaceutical Science*, 3(1), S93-S103. doi: 10.7324/JAPS.2013.34. S18

This reference also helped me analyze where lectins are found in nature, the role they serve, particularly in cell-cell recognition, mitogenic activity, cancer therapeutics and agglutination.

Statement of Progress

I have completed the coursework and have taken MLSC240 fundamentals of immunohematology that really give me the background and knowledge on blood banking and agglutination reactions. Additionally, I have the compounds that I will be using in order to test lectin activity.

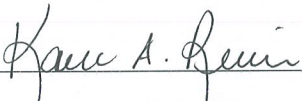
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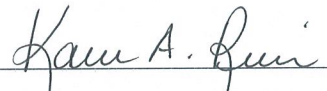
Department Chair Approval

- This student's performance in his/her major field is acceptable
- He/she has completed the requisite research methods coursework for the research to be pursued
- I understand that he/she plans to graduate with Honors


Department Chair (signature)

Research Advisor Approval

I have read and support this proposal


Primary advisor (signature)

If human subjects or if live vertebrate animals are involved, evidence of approval from the Institutional Review Board or an Animal Use Committee is needed through the campus scholarly research offices (Ext. 6361).