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Lectin Properties of Synthetically Produced Glucuronate, Alginate, and Related Boronates

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Abstract

In the nineteenth century, researchers discovered that some proteins had the ability to agglutinate red blood cells (Goldstein, 1980). These proteins were found mainly in the seeds of leguminous plants and were named phytohemagglutinins or hemagglutinins. Particular hemagglutinins were able to agglutinate red blood cells (RBCs) of a specific blood type. Now a days, these proteins are more widely known as Lectins. Lectins are proteinaceous macromolecules of nonimmune origin, capable of interacting with carbohydrates to form complexes (Goldstein, 1980). Lectins sources derive mainly from leguminous plants, animals, fruiting bodies of fungi, and bacteria. This research focuses on identifying the lectin activity and characteristics of certain synthetic compounds. Lectin activity was assessed by observing agglutination reactions of synthetic compounds and human red blood cells of a known human blood group and type in the ABO system. Lectins continue to be the focus of research due to their potential diverse applications specifically for blood grouping, mitogenic activity, and even stem cell transplantation.

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 19 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 human 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 the 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 type A human red cells 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 with sub-groups A. (Bird, 1959; Etzler & Kabat, 1970). Extracts from the asparagus pea, agglutinated type O human red cells but no other red cell 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) and their positive and negative health effects. 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 hinge 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 and aims to categorize the compounds according to their lectin-characteristic structures followed by their selective interaction with human red cells. These compounds have been previously screened for Lectin properties; however, specificity was within the scope of that previous study (Koshar, 2018).

Methodology

Background

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 is the absence of agglutination.

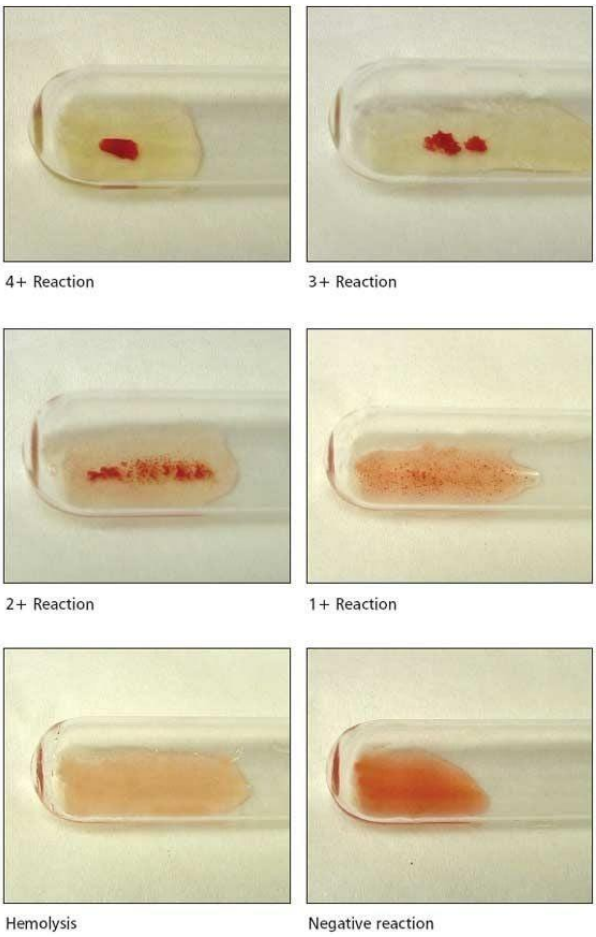

Figure 1. This figure shows the agglutination reactions from 4+ to a negative (0). A positive reaction raises awareness that there is significance and the laboratorian should be alert.

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 study was to first confirm previous findings reported on initial testing as reported by Koshar (2018), followed by specificity testing.

Testing Protocol

Type A, B, and O human red blood cells, from voluntarily donated units were used and five compounds were donated by the Andrews Chemistry department and Dr. Murray. Donated

blood is collected in bags containing acid citrate dextrose (ACD)-A, which contains 3% citrate to prevent coagulation and maximize cell life. Red cells for testing are prepared by washing the cells 3-4 times with 0.85% NaCl (isotonic saline). The washed red cells were then used to prepare a 2-5% red cell suspension in saline. The 2-5% red cell suspension was prepared as follows: To a labeled tube, I added 1 mL of whole blood. After I added the blood, I washed cells in saline for 5 minutes four times, removed last aspiration, and kept the pellet. Next, I added .3 mL of washed cells to another tube with 9.7 mL of saline. After adding washed cells with saline, I capped the tube with parafilm and shook it to obtain 2-3% red cell suspension or centrifuge for 15 seconds a total of 3-4 times. Finally, I added enough saline to achieve an adequate 2-5% red cell suspension.

A stock solution for each compound was prepared by adding 0.02 grams of each of the synthetically produced compounds to 1 mL of DMSO. The solution was mixed until dissolved.

Testing for hemagglutination was performed as follows:

1. Label 6 test tubes with respective compounds.
2. Add one drop of compound (ID) stock solution to each test tube.
3. Add 1 drop 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.

The tubes were examined macroscopically through grading of the tube using the standard grading method procedure on a scale of 4+-0. Additionally, I examined the tubes microscopically

through looking for clumping of cells with the aid of a microscope. Agglutination was observed through agglutination of cells, or clumping of cells.

Preparation of Controls

Controls for each stock solution were also prepared. However, initially there were some challenges in obtaining a suitable control. This had to do with DMSO, the solvent, causing the red cells to agglutinate. This reaction was unexpected because in previous studies (Koshar, 2018) this was not mentioned. To remediate this problem, a negative control was obtained by adding a 1:2 dilution of saline with DMSO as the control. It was necessary to have the DMSO and red cell suspension show no agglutination. This would allow the researcher to attribute any degree of agglutination to the compound and not the solvent. A negative control was achieved by diluting the DMSO with 0.85% NaCl.

A negative control was obtained with added compound and .5 ml of saline and .5 ml of DMSO. Additionally, a negative was also obtained with added compound and .5 ml of DMSO and .25 ml of saline. Thus, a negative control of .5 ml of DMSO and .25 ml of saline was used. Additionally, a prewarming technique was used in this experiment. First, the stock solution was heated at 37 degrees for 10 minutes as well as heating up each of the blood types. After heating up the stock solution and blood types, one drop of type A, B and O positive cells were placed in single tubes with stock solutions. At the end of the ten minutes, the tubes were centrifuged and read to record the results. The tubes were also read microscopically and macroscpically.

Results

Five synthetically produced compounds were used in this experiment: three glucuronic acid boronates and two acetylneuraminic acid boronates. The five compounds tested were 4-

bromomethyl phenyl boronic acid and glucuronic acid, 2-bromomethyl phenyl boronic acid and glucuronic acid, 3-bromomethyl phenyl boronic acid and glucuronic acid, 2-bromomethyl phenyl boronic acid and acetylneuraminic acid, and 3-bromomethyl phenyl boronic acid and acetylneuraminic acid. Solubility proved to be a problem; therefore, only two compounds were ultimately tested for lectin properties through agglutination. Solubility was a problem because only 2 compounds out of five were tested. Three compounds didn't dissolve originally with the DMSO and thus were not used in the experiment.

The two semi-soluble compounds were 4-bromomethyl phenyl boronic acid and glucuronic acid (compound 1) and 2-bromomethyl phenyl boronic acid and acetylneuraminic acid (compound 6). These two compounds were tested with type A Rh positive, type B Rh positive, and type O Rh positive human red blood cells to determine their hemagglutinin characteristics. Neither compound showed macroscopic agglutination. Compound 1 did not show microscopic agglutination for any of the red cell types. In contrast, compound 6 did show some microscopic reactions. When tested with A cells (Figure 2), no microscopic agglutination was observed. The results were similar with O cells (figure 4). However, when tested with B positive cells, a few microscopic clumps were observed (Figure 3).

Compound Name	Compound number	Used in the experiment
4-bromomethyl phenyl boronic acid and glucuronic acid	Compound 1	Yes
3-bromomethyl phenyl boronic acid and glucuronic acid	Compound 2	No

2-bromomethyl phenyl boronic acid and glucuronic acid	Compound 3	No
3-bromomethyl phenyl boronic acid and acetylneuraminic acid	Compound 4	No
2-bromomethyl phenyl boronic acid and acetylneuraminic acid	Compound 6	Yes

Figure 2. The above synthetic compounds describe the compounds that were used and not used in this research. In total, five compounds were donated by the chemistry department, but only two were used in the research because only two compounds were soluble enough to be tested.

Controls consisted of 0.5 mL of DMSO and 0.5 mL of normal saline along with each of three red blood cell types. All controls were negative for agglutination suggesting that any agglutination observed in the solution with the compound would be attributed to the compound.

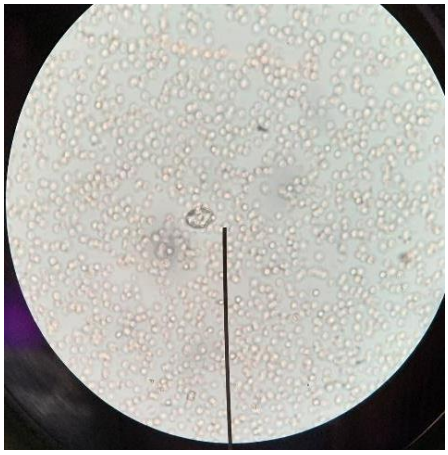

Figure 3. This figure shows compound 6 with A positive cells. There is no visible agglutination.

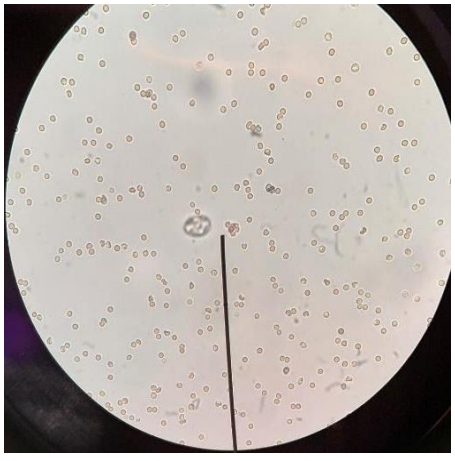

Figure 4. This figure shows compound 6 with B positive cells. There is slight agglutination in comparison with other cells.

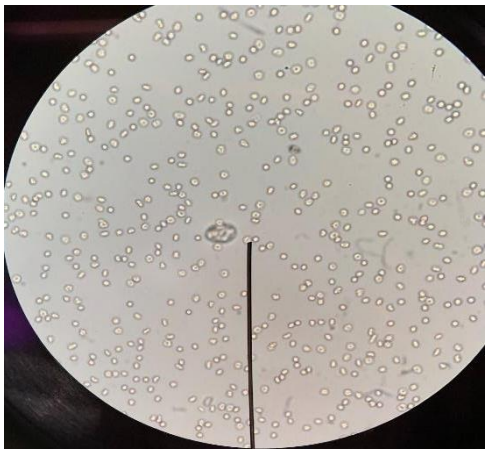

Figure 5. This figure shows compound 6 with O positive cells. Similar to type A cells, there is no visible agglutination.

Discussion

Five compounds were used in this experiment. However, solubility was a problem and only two compounds were successfully dissolved and thus used in this experiment. The two compounds were 4-bromomethyl phenyl boronic acid and glucuronic acid (compound 1) and 2-bromomethyl phenyl boronic acid and acetylneuraminic acid (compound 6). Compound 1 showed no hemagglutinin characteristics demonstrated by the lack agglutination with all three red cell blood types.

Although compound 6 did not show any agglutination with respect to macroscopic observations, compound 6 did show some microscopic observation. While there was no visible agglutination with A or O positive cells, compound 6 did show some slight hemagglutination with B positive cells. Compound 6 demonstrated some microscopic agglutination with group B Rh positive human red cells (figure 3). The agglutination was visible through some small clumps throughout the fields. For example, the pointer demonstrates a small visible clump of red blood cells after testing the red cells with 2-bromomethyl phenyl boronic acid and acetylneuraminic acid. These results suggest some selective hemagglutinin or lectin activity for compound 6.

The research purpose was to determine and analyze lectin properties in synthetically produced compounds. Lectins are used in the lab to help in ABO typing. The research showed that 2-bromomethyl phenyl boronic acid and acetylneuraminic acid showed slight agglutination with B positive red cells. This may mean that the compound could potentially be used as a lectin source in the laboratory for ABO typing.

This research did have some limitations and strengths. For example, one weakness that I had during this research was that I couldn’t test all the compounds because of solubility. Out of five compounds that were donated from the chemistry department and Dr. Murray, only two

compounds were tested. Further, the two compounds were semi-soluble. Additionally, it took awhile to find a good control for the testing. When the tubes were first tested with DMSO and red cells, the tubes agglutinated. Thus, a good control had to be sought in order to have validity in the actual testing.

Despite having some limitations in this research, there were also some strengths. For example, one strength was that a negative control was derived with a saline and DMSO ratio. The control included in this research was .5 ml of DMSO and .25 mL of saline. Additionally, another strength in this research was testing the two compounds with the red blood cells. Despite having some weaknesses and strengths, there was definitely potential for further research.

Further research could include testing compound 6 lectin activity on Rh negative red cells. This research focused on testing the compounds with A, B, and O positive cells. As a result, testing the same compounds with Rh negative red blood cells can indicate whether or not the compound is specific for Rh positive, Rh negative, or both. Additionally, because solubility was a challenge, it would be good to explore other types of organic solvents.

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