

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

Abstract

In the nineteenth century, researchers discovered the ability of some proteins to agglutinate red blood cells (Goldstein, 1980). The 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 particular blood type. 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 lectin properties of synthetically produced compounds through agglutination of human red blood cells. 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.

Methodology

A stock solution was prepared by adding 0.02 grams of compound to 0.5 mL of DMSO and 0.5 mL of 0.9% NaCl (normal saline).

The stock solution was

tested by adding one drop of stock solution and one of human red blood cells of known ABO Rh type to a 12x75 mm tube. After centrifugation, the tube was examined for agglutination.

Agglutinations strengths were graded as 4+ = solid clump; 3+ = several large clumps; 2+ = small to medium sized clumps with clear background; 1+ = small clumps with cloudy background; +w = tiny aggregates with cloudy background; + micro = positive upon microscopic examination only; hemolyzed (a positive reaction); neg = negative, no agglutination.

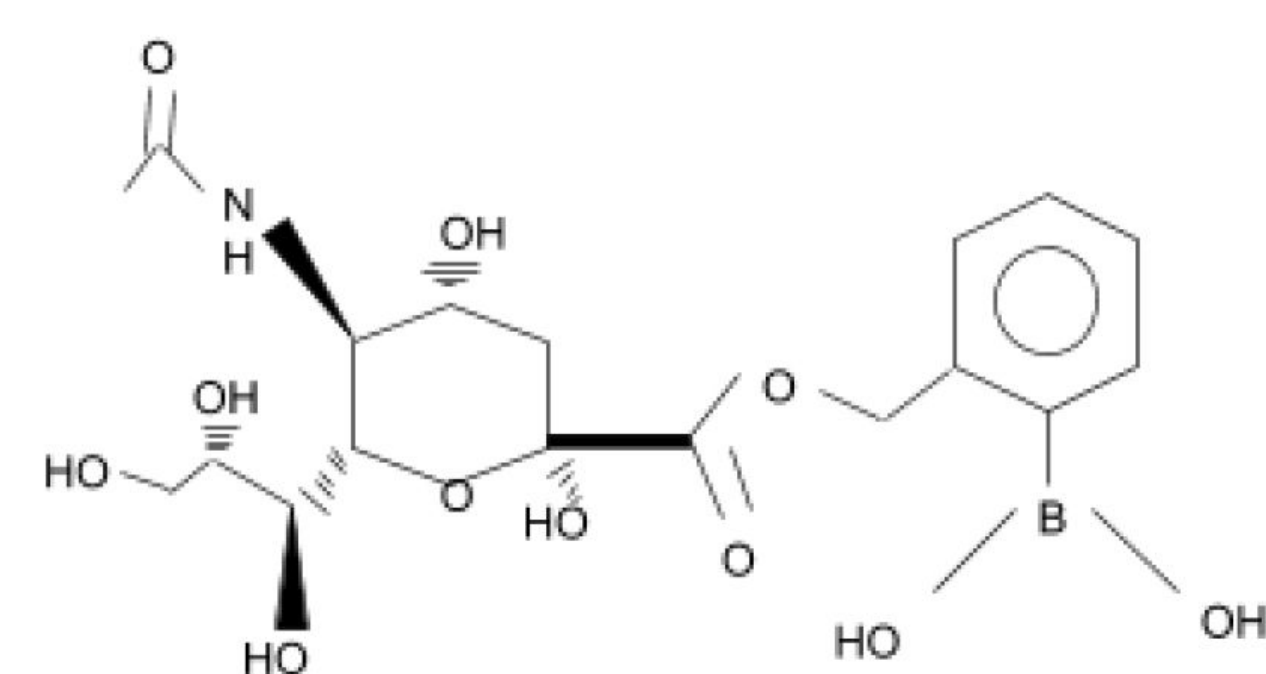


Figure 1. 2-Bromomethyl phenyl boronic acid and acetylneuraminic acid

Results

Five compounds were used in this experiment: three glucuronic acid boronates and two acetylneuraminic acid boronates. Solubility proved to be a problem; therefore, only two compounds were ultimately tested for lectin properties through agglutination. 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 cells to determine their hemagglutinins 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 cells a few microscopic clumps were observed (Figure 3).

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

Conclusion

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 4-bromomethyl phenyl boronic acid and acetylneuraminic acid (compound 6). Compound 1 showed no hemagglutinin characteristics demonstrated by the lack of agglutination with all three red cell blood types. Compound 6 demonstrated some microscopic agglutination with group B Rh positive human red cells (Figure 3). These results suggest some selective hemagglutinin or lectin activity for compound 6.

Further research could include testing compound 6 lectin activity on Rh negative red cells as well as other Rh phenotypes. Because solubility was a challenge, it would be good to explore other types of organic solvents.

Bibliography

- Dickers, K. J., Bradberry, S. M., Rice, P., Griffiths, G. D., & Vale, J. A. (2003). Abrin poisoning. *Toxicology Review*, 22 (3), 137-142.
- Fung, M. K., Eder, A., Spitalnik, S. L., & Westhoff, C. M. (2017). *Technical manual*. Bethesda, MD: AABB.
- Ghazarian, H., Idoni, B., & Oppenheimer, S. B. (2011). A glycobiology review: carbohydrates, lectins, and implications in cancer therapeutics. NCBIS. doi: 10.1016/j.jcthis.2010.02.004
- Gorakshakar, A., & Ghosh, K. (2016). Use of lectins in immunohematology. *Asian Journal of Transfusion Science*, 10(1), 12. doi: 10.4103/0973-6247.172180
- Koshar, E. (2018). Synthesis and Agglutinating-Coagulating Properties of Glucuronate, Alginate, and Related Boronates
- Lis, H & Sharon, N. (2004). History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*, 14(11). doi: 10.1093/glycob/cwh122
- 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

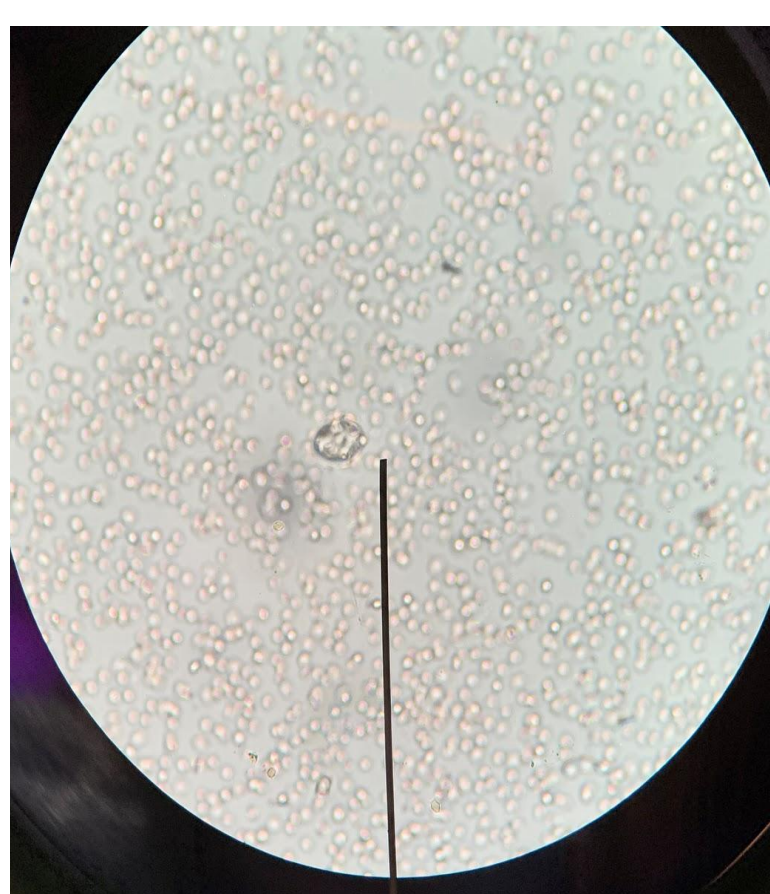


Figure 2. This figure shows A positive cells tested with compound 6. As observed microscopically, no agglutination is visible.



Figure 3. This figure shows B positive cells tested with compound 6. There are a few visible clumps displaying hemagglutinating properties. For example, the pointer has a clear small clump demonstrating agglutination microscopically.

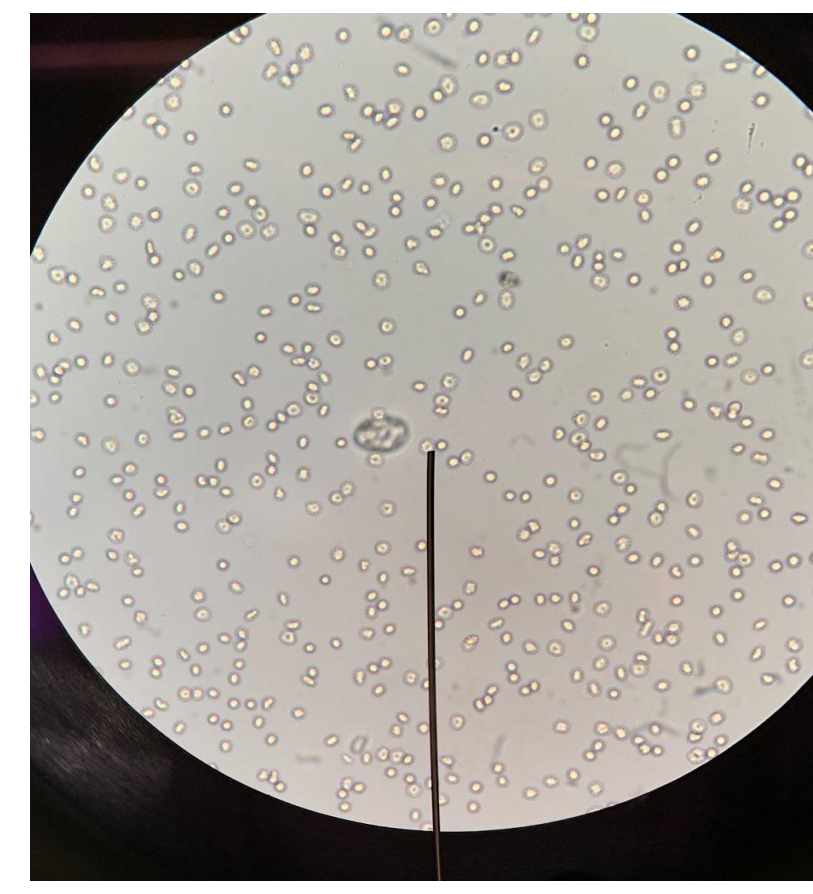


Figure 4. This figure shows O positive cells with compound 6. Similar to blood type A, Blood type O also shows no visible agglutination using the tube method neither did it show agglutination under the microscope.

