Chapter 6

The Use of Lectins (Agglutinins) to Study Cell Surfaces

Ingrith Deyrup-Olsen
Department of Zoology
University of Washington
Seattle, Washington 98195

PhD Columbia University, 1944; Instructor in Physiology, Columbia University, 1942–47; Assistant Professor to Professor, Barnard College, 1947–64; Research Professor to Professor, University of Washington, 1964– . Interests: Effects of histamine on circulation; water and electrolyte exchanges of mammalian tissues; amphibian water balance; water relations in gastropod molluscs.

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Introduction

Lectins are proteins which combine specifically with carbohydrate molecules, or with carbohydrate groups in such complex molecules as glycoproteins and glycolipids. Because carbohydrates are universally present on the outer surfaces of cell membranes, lectins can be used as tools to manipulate and mark cells at the level of their membranes. Experiments with lectins are easily carried out by students in elementary classes, and offer investigative challenges to students at more advanced stages. Probably the most valuable aspect of work with lectins is that it directs the student's attention to the diverse and crucial roles of the cell membrane in physiological processes.

In a familiar example of cell surface reactions, carbohydrate groups on the outer membrane of red blood cells give the basis for blood typing; plasma proteins (agglutinins—these are antibodies) react with the surface carbohydrates (agglutinogens) and clump the red cells, thus playing the role of lectins. Lectins which agglutinate red cells have been described in many plant tissues (for example, the seeds of beans, peas, lentils and other legumes) and in animal tissues (such as mammalian lung and liver, and blood cells and egg fluids of certain molluscs). Indeed, as more and more studies are carried out, the distribution of lectins begins to seem nearly universal among higher organisms. Lectin-carbohydrate reactions may be involved in cell-cell interactions such as those basic to tissue organization; in egg recognition by sperm; and in defense reactions. Lectins, such as the plant product *concanavalin A,* can stimulate cell division, apparently by triggering control processes at the cell membrane. As experimental tools, lectins labelled with fluorescent dyes or with radioactive atoms can attach to cell suface carbohydrates and then serve as markers for changes occurring in the cell membrane during such processes as ameboid movement, phogocytosis, and cell division.

Depending on the instructor's objectives, lectins may be used by students in many different ways. Typically, a crude preparation of plant or animal material can be prepared by students in a few minutes. Agglutination of cells (red blood cells, yeast, protozoans, etc.) can be observed within a few minutes to an hour or so; thus, laboratory procedures can be compressed into a short (e.g., 2 hour) period. In the experiment outlined in the following paragraphs, the student should:

1. prepare a biologically active material, containing a lectin, from plant tissue;
2. observe the clumping (agglutination) of cells by the lectin;
3. estimate the activity of the extract by the method of serial dilution.

Instructors' Materials

Outline of Procedures

1. Using a mortar and pestle, grind up about 1 gm of plan tissue (potato tuber, lentil seeds, or other) in 2 ml of 0.15 M (0.875%) sodium chloride solution. Filter or centrifuge the mixture to prepare the lectin-containing fluid (which is cloudy, but freed of large particles).
2. Prepare a red cell suspension: with a sterile lancet, collect a small drop of blood (finger tip prick) and place it in the upper end of a test tube containing 1 to 2 ml of 0.15 M NaCl. Quickly stopper the test tube and invert it several times to mix the blood with the NaCl solution (thus preventing clotting of the blood).
3. Mix a drop of (1) with a drop of (2) in a depression slide, and stir with a tooth pick. Observe at intervals with a hand lens or microscope (at about 100 x). A control sample containing a drop of (2) and a drop of 0.15 M NaCl should be observed as well.
4. Prepare dilutions of the plant tissue extract (e.g., 1 part plant extract diluted ½, ¼, ⅛ etc. with 0.15 M NaCl) and find the greatest dilution at which clumping of cells still occurs. (This procedure can be used to compare different plant tissues for the presence of lectins, or to compare cells from different sources for their reactivity with a given lectin).

Additional Notes

Specific lectins react with different sugars, and a "lectin dictionary" is being built up to "read" the information coded on cell surfaces by their characteristic carbohydrates. (As an example of this approach, the chemical supply company P-L Biochemicals lists about 20 purified lectins, giving the unique carbohydrate specificity of each). Human red blood cells of types O, A, B, and AB are all clumped by the potato lectin, but lectins extracted from kidney beans and lima beans selectively clump type A cells only. A widely used lectin is concanavalin A (isolated from seeds of the jack bean, *Conavalia ensiformis*). It clumps the blood cells of many species, including those of rats and mice, but does not clump human red blood cells. Interestingly, in some cases in tissue culture, cells which have undergone malignant transformation (e.g., following virus infection) are agglutinated by concanavalin A, while normal cells are not. Advanced students may find it interesting to explore cell surface specificities using lectins and blood cells from several sources.

The evidence that lectins bind specific carbohydrate groups on surface membranes comes from the observation that when particular sugars are present in mixtures of blood cells with lectin, agglutination is *inhibited*. For example, clumping of blood cells by concanavalin A is blocked by the simple

sugars glucose and mannose. Students can test the effects of different sugars on agglutination by the lectin with which they are working.

Since lectins are proteins they can be inactivated by procedures which denature or break down proteins, such as heating, extremes of pH (from 3 to 4 and from 8 to 9), and proteolytic enzymes. In some cases lectins are dependent on the presence of particular ions. Interesting results follow the reduction of calcium ion concentration to approximately zero using 20 mM EGTA, or raising $[Ca^{++}]$ to 20 to 30 mM. Students can explore the properties of the lectin with which they are working from these points of view. A further possibility for investigation is the changing pattern of lectin content with development (would a germinated bean seed or a young bean plant contain lectin in amounts similar to the dormant seed?)

In our laboratories we have used lectin-cell interactions chiefly as a basis for independent projects for intermediate-level and advanced students. There are many directions in which students can elect to explore on their own, since this a relatively new area with numerous questions still open. For the elementary student the study of cell-lectin reactions could make the complex structure of the cell membrane more understandable and concrete, and extend its study well beyond the common and limited approach of observing osmotic phenomena alone.

References

Barondes, S. H. Lectins: their multiple endogenous cellular functions. Ann. Rev. Biochem. 50:207–231; 1981. *An excellent, recent review of general aspects of lectins.*

Cohen, E., editor. Biomedical perspectives of agglutinins of invertebrate and plant origins. Ann. N. Y. Acad. Sci. 234: 1; 1974. *This reference is valuable as a rich source of background information and ideas.*

Sharon, N. Lectins. Sci. Am. 236(6):108–119; 1977. *This is a brief, readable introduction to lectins, and can be used as a direct reference for students.*

Sharon, N.; Lis, H. Lectins: Cell-agglutinating and sugar-specific proteins. Science 177:949–959; 1972. *Similar to the above review, but more detailed.*

APPENDIX A
Sources of Red Blood Cells

Red blood cell suspensions may be prepared in advance by the instructor. For example, a rat is anesthetized surgically with ether, and about 1 ml of blood is drawn by heart puncture into a heparinized syringe. (This procedure is easily tolerated by the rat, which should recover fully in a few days). The blood is mixed in a plastic centrifuge tube with about 10 ml of 0.15 M NaCl, and the mixture is centrifuged for 5 to 10 min in a clinical centrifuge at about 5000 rpm. The supernatant is pipetted off and discarded, the cells are resuspended in 0.15 M NaCl, and the mixture is again centrifuged. This washing procedure removes most of the plasma proteins; these may otherwise inhibit cell-lectin interactions. After a second washing the cells are resuspended in 25

ml of 0.15 M NaCl to make a 2% suspension, which should provide enough material for 50 students working in pairs). The cell suspension is stable in the refrigerator (4°C) for several days. Samples of human blood larger than finger prick samples may be obtained by venipuncture through cooperation of the health or physician's office. The instructor should not offer to carry out this simple but illegal procedure. The use of spent blood-bank blood can no longer be considered safe. Blood cells from fish, amphibians, birds and reptiles may be investigated profitably by students. The general methods for sampling and washing of cells outlined above may be used, with appropriate adjustments of the tonicity of NaCl solutions for the vertebrate class investigated.

APPENDIX B
Commercial Sources of Lectins and Related Compounds

Lectins can be purchased in highly purified form from biochemical supply companies such as:

Sigma Chemical Co.
P.O. Box 14508
Saint Louis, Mo. 63178

P-L Biochemicals, Inc.
1037 West McKinley Ave.
Milwaukee, Wi. 53205

Lectins tend to be very expensive, and some are highly toxic. Since lectins are extremely powerful agents, analogous in this as in many other respects to enzymes, small quantities go far in the teaching laboratory. In our teaching situation we find it useful to have on hand small quantities of concanavalin A and blood typing sera, which students can use as comparative substances in their own investigations.

The sugars which competitively inhibit cell-lectin reactions (e.g., glucose, mannose, fucose, N-acetyl glucosamine, N-acetyl galactosamine) can be purchased from the suppliers listed above as well as from general chemical supply houses. The chelating agents EGTA and EDTA both bind Ca^{++}. EDTA also binds Mg^{++}. Both are available from the above suppliers.

APPENDIX C
Yeast

Yeast is readily obtained in grocery stores and supermarkets. A gram or so of the commercial preparation is suspended in about 500 ml of 0.1 M phosphate buffer, pH 7, + glucose or sucrose (added to give a concentration of about 2%). The mixture is maintained for a few days at room temperature; additional sugar (about 1 gm per day) is added to foster vigorous growth of the organisms. Shortly before use the suspension is centrifuged; the sedimented yeast cells are resuspended in phosphate buffer, the supernatant is discarded, and the cells are resuspended in phosphate buffer. This washing procedure is repeated 2 or 3 times to remove exogenous carbohydrates (which could inhibit the lectin reaction), and the cells are finally suspended in phosphate buffer. Concanavalin A ("ConA") agglutinates yeast cells. Once students have observed agglutination, they may test a variety of biological materials to search for lectins which recognize yeast cell wall carbohydrates. Alternatively, they may test for competitive inhibition of ConA agglutination, varying factors (pH, ionic composition) of the medium, or other ways in which agglutination may be altered.

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