THE INHIBITION OF THE PEANUT-AGGLUTINATION REACTION IN STREPTOPELIA RISORIA BY SIMPLE SACCHARIDES

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Introduction

Human erythrocytes are known to be specifically agglutinated by certain lectins or plant agglutinins. Lectins have been found to specifically agglutinate in the ABO system. In 1959 Boyd, Green, Fujinaga, Drabik and Waszczenko-Zacharczenko went beyond using lectins to detect only known blood factors, and reported a possible new blood factor detected while testing human red blood cells with extracts from Arachis hypogaea, the common peanut. These workers then conducted inhibition tests with simple sugars on this factor to determine a possible receptor on the red blood cell for this new lectin factor.

In 1965, Miller published a report on a lectin factor in doves that is controlled by a recessive gene. The lectin used was again Arachis hypogaea. No work had been done to see what saccharides will inhibit the lectin agglutination with red blood cells of doves and hence, point to a possible receptor on the dove erythrocyte. The following is a report of a quarter's study, completed by the writer on: (1) The inhibition by simple sugars of this recessively controlled lectin factor, (2) The probable structure of the peanut receptor on the dove erythrocyte, and (3) The unlikelihood of a correlation between receptors on the human erythrocyte and that of the dove erythrocyte.

Materials and Methods

Peanut Extract

The lectin extract used was from Arachis hypogaea. This extract was prepared by Dr. Miller as reported in his paper of 1965. The lectin extract was used at a dilution of 1/4, sufficient to give total agglutination with the red cells in the lectin control.

Red Cell Procedure

Doves used in tests were from the ringneck dove colony maintained at Iowa State University by the Department of Genetics, and under the care of Dr. Wilmer J. Miller. Only mature birds of known peanut reactivity or non-reactivity were used. Several were used as test control birds.

Bleeding of the birds was accomplished by two methods -- bleeding from the wing vein and bleeding directly from the heart. Sodium citrate anticoagulant was used.

Red cells were stored in their own plasma until used. Test cells were made by packing and washing in centrifuge tubes -- each

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of three to four washings with fifteen volumes of isotonic saline solution (.92%). After the last washing was made and the supernatant drawn off, a standard three percent red blood cell suspension was made in a graduated centrifuge tube with the isotonic saline.

Sugar Solutions

Sugar solutions were made by weighing sugars into .004M and .003M portions, then dissolving them into 2 and 3 ml. volumes to form 2 M and 1 M solutions respectively. A .5 M solution was made by making a quadrupling dilution of the 2 M solution. From the 1 M solution 1/4 M, 1/16 M, 1/64 M, and 1/256 M solutions were made by making continuing quadrupling dilutions. Some sugars, because their solubility saturation points were less than 1 M, were made in 1/4 solutions as the highest concentration used in the tests; fortunately, for all such sugars, inhibition occurred at concentrations of 1/64 M. Sugars were then stored frozen, after being used in one test, for later tests. A test with a lactose solution -- made, stored frozen, and then used in a test, compared to another test with a lactose solution made the same day, gave the same results as the test using the lactose solution made on the test date.

Method of Agglutination

One drop of the diluted sugar as inhibitant (in rows of decreasing concentrations) was added to one drop of peanut extract in each of the wells of the perspex plastic plates, which were used in all tests. After an incubation period of fifteen minutes at 25-26° C. the red blood cell suspensions were dropped. Equal volumes of peanut extract, sugar solution, and red blood cell suspension were then in each well of the plate.

Three readings were taken, the first at five minutes, the second at half an hour, and the last at two hours.

Results

Test results of each sugar are generalized and summarized in Table I. All readings are for those at two hours. (L.C. = Lectin Control) The weakest concentration of inhibition was determined by the criterion, that if the cells of two birds showed a concentration as being last where inhibition occurred, then this concentration was used in the chart. Example: If the red cells of bird "A" were inhibited to 1/16 and bird "B" to 1/4, for a sugar, the concentration used in the chart would be 1/16, for both showed agglutination at this concentration. (This variation was infrequent; but when it occurred, that procedure was used.) From this table, those sugars tested may be divided into three very simple groups.

Group I. Best inhibitors - inhibited to dilutions of 1/64 M or .0156 M

1. α - Cellobiose
2. D - Galactose
3. Lactose
4. Raffinose

Group II. Mediocre inhibitors - inhibit to dilutions of 1/4 M to 1/2 M, or .25 M to .50 M

1.	L (+) Arabinose	1/2
2.	D-Glucose	1/2
3.	Maltose	1/4
4.	D-Mannose	1/2
5.	α-D-Melibiose	1/4
6.	L-Rhamnose	1/2
7.	Sucrose	1/2
8.	Trehalose	1/2

Group III. Poor inhibitors - uninhibitory or nearly so

1.	D-Mannitol	2 M
2.	D-Sorbitol	2 M
3.	D-Xylose	2 M
4.	D(-) Arabinose	None
5.	D-Fructose	None

The three generalized groups could be divided, of course, into more specific groups, but as there is no apparent necessity for this and there are two rather sharp divisions, it was not done.

Discussion

Inhibition of the peanut agglutination reaction, by the saccharides used, indicates that the peanut factor is a physical entity on the dove erythrocyte. The peanut factor will most probably have a very exact structure, but the test results cited can only give indications as to the immunochemical structure involved.

Studying the three groups of saccharides in a general overview, one notices that there is a positive correlation between sugar complexity and inhibitory power. Only monosaccharides are found in the poorest inhibiting group. In the middle inhibiting group, monosaccharides are found in equal numbers with disaccharides. In the best inhibiting group, there are two disaccharides (lactose and cellobiose) and a trisaccharide (raffinose).

One can propose a general statement of the peanut lectin specificity of the dove erythrocyte. It will accept a galactose-like unit to give agglutination. The test results are very exclusive in a positive manner, i.e., if a saccharide contained a molecule of galactose then it was a good inhibitor.

Other saccharides inhibit to a moderate extent. Cellobiose, already mentioned, inhibits very well. A possible explanation for alpha-cellobiose's good inhibitory powers is that, cellobiose contains a beta linkage between glucose and glucose, noting that a beta linkage of glucose and another molecule correlates with good inhibition.

All the test results cited here indicate that the peanut receptor is very similar to the receptor on the human red cell. Boyd in reporting the "Gy" human blood factor found that galactose and oligosaccharides containing galactose inhibited the human peanut reaction. Boyd, finding that glucose has inhibitory powers and that cellobiose, a glucose-glucose disaccharide, and maltose, a glucose-alpha-glucoside,

inhibit very well suggests that glucose is the next-to-terminal unit in the peanut receptor.

Glucose is not obviously the next-to-terminal unit on the dove erythrocyte, yet it is the most likely possibility, due to its effective inhibition when with the beta linkage.

The results suggest that the apparent medium inhibition titers of the dissaccharides in this group could be due to their alpha linkage or complexity. Glucose itself is in this group - but so are three other distinct monosaccharides. It is most probable then that the appearance in this group of the disaccharides, maltose, melibiose, sucrose and trehalose, containing glucose is due, not necessarily to the glucose, but to the more complex structure of the disaccharide. Findings by Weber, (unpublished), show that after absorptions of peanut extract by human positive red cells there is still nearly as strong an agglutination reaction in titer with dove peanut positive cells. Hence, one can be sure that the structure of the lectin factor on the dove red blood cell is chemically different from the lectin factor on the human red blood cell in spite of the closely similar sugar inhibition results.

Conclusion

Test results point to a galactose structure on the terminal unit of the dove erythrocyte lectin receptor. If there is a next-to-terminal or at least another important unit, results allow the implication, but do not prove that it is not glucose. Test results indicate that there may be any number of units on the dove red blood cell with which the peanut lectin may combine, but the results are not conclusive and detailed enough to tell what these units are. Cellobiose is the biggest question mark in the test results. One possible explanation is that inhibition seems to follow glucose and a beta linkage or galactose and alpha linkage with perhaps a glucose as the other molecule.

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TABLE I.

Results of Inhibition of Agglutination
Tests on P+ Birds

Saccharide	Titer Strength in Moles							
	2	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{256}$	LC
D(-) Arabinose	++	1	2	4	4	4	4	4
L(+) Arabinose	.	.	++	1	1	2	3	4
Alpha - Cellobiose	.	.	.	.	.	++	3	4
D-Fructose	±	1	1	2	4	4	4	4
D-Galactose	.	.	.	.	.	++	2	4
D-Glucose	.	.	±	++	2	3	4	4
Lactose	.	.	.	.	.	±	2	4
Maltose	.	.	.	±	2	4	4	4
D-Mannitol	.	++	1	2	3	3	4	4
D-Mannose	.	.	1	2	3	3	4	4
Alpha-D-Melibiose	.	.	.	±	++	1	2	4
Raffinose	.	.	.	.	.	1	2	4
L-Rhamnose	.	.	++	2	2	3	4	4
D-Sorbitol	.	++	1	2	4	4	4	4
Sucrose	.	.	±	1	3	4	4	4
Trehalose	.	.	.	±	2	4	4	4
D-Xylose	.	1	1	2	3	4	4	4

4 = total agglutination
. = no agglutination
other symbols = intermediate strengths of agglutination