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INHIBITION OF BROAD SPECTRUM HEMAGGLUTININ FROM *PSEUDOMONAS AERUGINOSA* BY D-GALACTOSE AND ITS DERIVATIVES

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1. Introduction

Hemagglutinins from animal, plant, fungal, bacterial and viral origins have been described [1–5]. Their specificity is manifested in the spectrum of animal cells which they agglutinate and in their inhibition by certain carbohydrates [1, 2]. This specificity provides a unique opportunity for the investigation of the biochemical nature of the combining sites on cell membranes and other biological materials. The blood group specific hemagglutinins are inhibited by the sugars that determine the blood groups antigens [1, 2, 6, 7] and the broad spectrum hemagglutinins are inhibited by different sugars. The phytohemagglutinin of *Phaseolus vulgaris* is inhibited by *N*-acetyl-D-galactosamine [1, 2, 8]; the wheatgerm hemagglutinin is inhibited by terminal non-reducing *N*-acetyl-D-glucosamine [9]; concanavalin A of *Canavalia ensiformis* is inhibited by α-methyl-D-glucopyranoside [10] and by various α-glucans [11, 12]; the soybeans hemagglutinins are inhibited by *N*-acetyl-D-galactosamine and by D-galactose [13–16]; the phytohemagglutinin of *Pisum sativum* is inhibited by D-mannose, D-glucose and D-fructose [17] and the hemagglutination due to fava beans is inhibited by D-glucose, D-fructose and maltose [18].

In the present study, bacterial hemagglutinin (BHA), separated from *Pseudomonas aeruginosa* cells, was found to exhibit a broad spectrum cell specificity, to agglutinate preferentially papain or neuraminidase-treated erythrocytes and to be eluted from the cells by heating at 56–70° or by acidification. The hemagglutination induced by the BHA was inhibited by D-galactose, *N*-acetyl-D-galactosamine and lactose.

2. Materials and methods

A pyocyanin-producing strain of *P. aeruginosa* was grown in nutrient broth (Difco) with shaking at 28° for 3 days. The growth medium was provided daily with 0.2% acetylcholine instead of glucose. The cells were harvested, washed 3 times in an isotonic saline and suspended to obtain a 20% suspension. The cell suspension was exposed to Raytheon 10 KC 250W sonic oscillation for 5 min and centrifugated at 30,000 *g* for 15 min. The supernatant fluid, containing the bacterial hemagglutinins, was kept at −20°.

Human and animal bloods were taken into one fifth of their volume of 3.8% sodium citrate solution, the erythrocytes were separated and washed 3 times in saline. Papain-treated erythrocytes [19] were prepared by incubation of 9 vol of 5% suspension of washed erythrocytes with 1 vol of 1% papain (crude preparation, Sigma) in 0.1% cysteine solution at 37° for 30 min. Neuraminidase-treated erythrocytes were prepared by incubation of 9 vol of the 5% erythrocyte suspension with 1 vol of neuraminidase solution (Serva preparation, which removes 500 μg of *N*-acetyl neuraminic acid in 15 min at 37°) in 0.05 M acetate buffer at pH 5.5 containing 0.01 M $CaCl_2$. The enzyme-treated cells were washed 3 times in saline.

Hemagglutination tests were performed by addition of 0.05 ml of 5% erythrocyte suspension (enzyme

Table 1
Hemagglutination of untreated and enzyme-treated human and animal erythrocytes by the BHA preparation.

Erythrocytes	The BHA hemagglutination titer											
	1	2	3	4	5	6	7	8	9	10	11	12
untreated:												
Human	++++	++	+	–	–	–	–	–	–	–	–	–
Rat	++++	++++	+++	+	–	–	–	–	–	–	–	–
Rabbit	+	–	–	–	–	–	–	–	–	–	–	–
Dog	++++	++++	+++	++	–	–	–	–	–	–	–	–
Mouse	++	+	–	–	–	–	–	–	–	–	–	–
Sheep	+	–	–	–	–	–	–	–	–	–	–	–
Neuraminidase treated:												
Human	++++	++++	++++	++++	++++	+++	++	++	+	–	–	–
Papain treated:												
Human	++++	++++	++++	++++	+++	+++	++	++	++	+	–	–
Rat	++++	++++	++++	++++	++++	++++	++++	+++	++	++	+	–
Rabbit	++++	++++	+++	+++	++	++	++	++	++	+	–	–
Dog	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	++	+
Mouse	++++	++++	++++	++++	+++	+++	+++	+	–	–	–	–
Sheep	++++	++++	++++	++++	+++	++	++	++	+	+	–	–

The numbers represent a series of 2-fold dilutions of the BHA preparation beginning from 1/40. The results presented are an average of 2–10 tests.

Table 2
Hemagglutination of papain-treated human erythrocytes by BHA preparations which were incubated with different sugars.

The sugar added to the BHA	The BHA hemagglutination titer										
	1	2	3	4	5	6	7	8	9	10	11
None	++++	++++	++++	++++	++++	++++	++++	+++	+++	++	+
D-Glucose	++++	++++	++++	++++	+++	++	++	++	+	+	–
D-Galactose	–	–	–	–	–	–	–	–	–	–	–
D-Fructose	++++	++++	++++	++++	+++	+++	+++	+++	++	+	–
D-Mannose	++++	++++	++++	++++	+++	+++	+++	++	+	–	–
N-Acetyl-D-glucosamine	++++	++++	++++	++++	++++	++++	++++	+++	++	++	–
N-Acetyl-D-galactosamine	++	++	++	++	++	+	–	–	–	–	–
Lactose	++	++	++	+	+	±	±	±	±	±	–

The numbers represent a series of 2-fold dilutions of the BHA preparation beginning from 1/40.

treated or untreated cells) to serial 2-fold dilutions of the BHA preparation in 0.2 ml vol of saline, incubation for 1 hr at room temp and a very short centrifugation. The degree of the agglutination was graded on a (–) to (++++) scale.

Elution of the agglutinins from the cells was conducted by washing them 3 times and either suspending them in saline and heating at 56–70° for 10 min, or suspending them in saline containing 3.3% acetic acid with shaking for 10 min, centrifugation and neutralization of the supernatant fluid.

Inhibition of the agglutinating activity of the BHA by different sugars was examined by incubating 0.2 ml of the BHA preparation, diluted 1/20, with 0.2 ml of a solution containing 10 mg of the examined sugar at room temp for 1 hr before titration of its hemagglutinating activity.

3. Results and discussion

The BHA from *P. aeruginosa* was separated from the bacterial cells by means of sonic vibrations and centrifugation. The subcellular preparation was found to resemble the broad-spectrum phytohemagglutinins in its activity. It agglutinated human red blood cells of different ABO, Rh and MN blood groups as did intact Pseudomonas bacteria [4]. The BHA agglutinated also the erythrocytes from various animals, including rats, rabbits, dogs, mice and sheep. The titer of agglutination of papain or neuraminidase treated cells was always much higher than that obtained for untreated cells, resembling the behaviour of "incomplete" antibodies and certain phytohemagglutinins [1, 2, 8, 14]. These results are presented in table 1.

Heating at 56° and acidification of the medium, which induce elution of antibodies and phytohemagglutinins from agglutinin-coated cells, also eluted the bacterial hemagglutinins. Higher temperatures (up to 70°) could also be employed for elution of the relatively heat resistant BHA, which resemble phytohemagglutinins and not antibodies in this respect.

The hemagglutination by BHA was found to be strongly inhibited by D-galactose, *N*-acetyl-D-galactosamine and lactose. A very slight inhibition was observed with some of the other sugars examined (table 2). *N*-Acetyl-D-galactosamine also inhibits the hemagglutinating activity of some broad spectrum phytohemagglutinins including those of *P. vulgaris* and soybeans [14]. The BHA differ from Phaseolus hemagglutinins [1, 2, 8] in being strongly inhibited by D-galactose.

The biological significance of the reactions between bacterial hemagglutinins and certain sugars and cell receptors, which are similar to those of certain phytohemagglutinins, is not understood.

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