A STUDY OF THE AGGLUTINATING PROPERTIES OF PHYTOHEMAGGLUTININ OBTAINED FROM SAX VARIETY OF Phaseolus vulgaris

M. T. Tsoneva-Maneva and E. P. Ugryumov

Department of General Biology (Head Professor, R. P. Popivanov), Superior Medical Institute, Sofia, and Institute of Experimental Biology (Director Professor, I. N. Maiskii), Academy of Medical Sciences of USSR, Moscow
(Presented by Acting Member of the Academy of Medical Sciences of USSR N. N. Zhukov-Verezhnikov)
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The presence of phytohemagglutinins in different plant species was determined early in this century. Especially frequently and in considerable amounts they are found in the seeds of representatives of the family Leguminosae [1, 2, 8, 14]. The hemagglutination properties of these substances, produced naturally by plants, have a wide range, but they are usually non-specific.

A specific action of phytohemagglutinins was noted in a number of cases [3, 5, 6]. The attention of the investigators was attracted toward phytohemagglutinins obtained from Phaseolus vulgaris and P. communis. The latter are characterized by a high phytohemagglutinin concentration in their seeds and by an absence of a toxic effect [6]. These phytohemagglutinins are used in experimental medicine for the purpose of separation of erythrocytes and leucocytes from whole blood [9].

Phytohemagglutinins are also of considerable interest in the chemotherapy of tumors. For example, it has been shown that certain phytohemagglutinins had an inhibiting effect on tumor cells in vitro [7, 13, 10].

During recent years it has been established that phytohemagglutinins of P. vulgaris stimulate in vitro the mitotic division of white blood cells from human peripheral blood [12]. This discovery had permitted the development of a comparatively simple method for the establishment of short-term cultures of human white blood cells from peripheral blood; this has opened wide possibilities for the normal and pathologic cytogenetic study of man [11, 12].

The phytohemagglutinin used for this purpose is chemically a mucoprotein, which at low pH values of the medium dissociates into two components: a protein and a polysaccharide. The protein component is biologically active while the polysaccharide has no agglutinating properties [4, 15, 16].

Our problem has been to obtain a chemically pure phytohemagglutinin preparation from P. vulgaris (Sax var.) and to study its biological properties.

EXPERIMENTAL METHOD

Seeds of P. vulgaris (Sax var.) were used to obtain the phytohemagglutinin. We have used the method of Rigas and Osgood [16] in order to obtain a pure phytohemagglutinin protein (P-PHA).*

The extraction was made in 1 N HCl from powdered seeds. The extract was purified by treating it twice with ammonium sulfate and then by dialyzing it twice against tap and distilled water. According to Rigas and Osgood [16], this yields a chemically pure phytohemagglutinin protein in the amount of 2 g per 1 kg of seeds. P-PHA is insoluble in distilled water but is easily soluble in a 0.85% solution of NaCl. The preparation may be obtained in the amorphous state by means of precipitation in distilled water, ultra-centrifugation and a subsequent lyophilization.

*This method is used in production, and by the firm Difco for the production of their preparation Bacto-phytohemagglutinin (Catalog No. 0528).

Results of Study of Agglutinating Properties of Phytohemagglutinin from Phaseolus vulgaris (Sax Variety)

Subject investigated		Dilution of phytohemagglutinin											
		1 : 1	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024	Control
Erythrocytes	Human A-group	++++	++++	++++	++++	++++	++	+	—	—	—	—	—
	Human B-group	++++	++++	++++	++++	++++	+++	++	—	—	—	—	—
	Human O-group	++++	++++	++++	++++	++++	++++	+±	±	—	—	—	—
	Rabbit	++++	++++	++++	++++	±	—	—	—	—	—	—	—
	Mouse	++++	++++	++++	++++	++++	++++	±	—	—	—	—	—
	Rooster	+++	+++	+++	+++	+++	+++	—	—	—	—	—	—
	Frog	+++	+++	++++	++++	++++	+++	±	—	—	—	—	—
		+++	+++	+++	+	—	—	—	—	—	—	—	—
		+++	+++	+++	+	±	—	—	—	—	—	—	—
Strains of normal cells	Amnion	++++	++++	++++	++++	++	+	±	—	—	—	—	—
	Skin epithelium	++++	++++	++++	++++	+++	+++	±	—	—	—	—	—
		++	+	+	±	—	—	—	—	—	—	—	—
	Fibroblasts	+++	++	++	++	—	—	—	—	—	—	—	—
		++++	++++	++++	++++	++++	++	—	—	—	—	—	—
		++++	++++	++++	++++	++++	+++	—	—	—	—	—	—
Strains of malignant cells	Human stomach cancer	++++	++++	++++	++++	++++	++	±	—	—	—	—	—
		++++	++++	++++	++++	++++	+++	++	++	—	—	—	—
	Human uterine cancer	+	±	—	—	—	—	—	—	—	—	—	—
		+	+	+	—	—	—	—	—	—	—	—	—
	Human larynx cancer	++	++	+	+	±	—	—	—	—	—	—	—
		++++	+++	++	++	±	—	—	—	—	—	—	—
	Mouse cancer	++++	++++	++++	++++	+++	++	—	—	—	—	—	—
		++++	++++	++++	++++	+++	++	—	—	—	—	—	—
	Rat cancer	+++	+++	+++	+++	+++	+++	±	—	—	—	—	—
		+++	+++	+++	+++	+++	+++	±	—	—	—	—	—
Bacteria	B. coli C-85	—	—	—	—	—	—	—	—	—	—	—	—
	B. coli K-12	—	—	—	—	—	—	—	—	—	—	—	—
	B. prodigiosum Strain 10	—	—	—	—	—	—	—	—	—	—	—	—

We have obtained P-PHA as a suspension in distilled water, as a viscous and a strongly opalescent fluid. By adding an appropriate amount of NaCl, the concentration of the latter was brought up to 0.85%, after which P-PHA dissolved completely. We used the preparation at this stage in our experiments. The concentration of P-PHA in the preparation, considering the original amount of seeds and the data of Rigas and Osgood, was equal to approximately 0.0005 g (500 μg) per 1 ml.

The study of the agglutinating properties of this preparation was made on the following subjects:

1. Human erythrocytes with A, B and O antigens, as well as erythrocytes of rabbit, mouse, rooster, and frog.

2. Human cells from monolayer serial cultures: amniotic (strain A-1), normal skin epithelium (strain 580) and fibroblasts (strain 558).

3. Tumor cells from monolayer serial cultures: human stomach cancer (Cave strain), human uterine cancer (HeLa strain) and human larynx cancer (strain HEp-2), as well as tumor cells of Ehrlich's mouse adenocarcinoma (ascitic form) and cells of ascitic tumor from rat ovary (strain OR).

4. Intestinal bacteria: B. coli C-85, B. coli K-12, B. prodigiosum (strain 10), all normal according to their cultural, morphological and biochemical characters.

In order to study the hemagglutinating properties of our preparation on the different kinds of erythrocytes, we diluted it in Vidal's tubes with 0.85% NaCl in concentrations of 1:1 to 1:1024. We used 5% erythrocyte suspensions. The cells were washed three times. To each drop of a corresponding dilution of the preparation we then added a drop of the erythrocyte suspension. The results were read twice: 1 hour after keeping the preparation at room temperature (20°C) and 24 hr after refrigeration at 4°C.

In experiments with material from monolayer cultures and from ascitic tumors we have used cell suspensions prepared in the following manner. Cells were removed from glass with a 0.2% solution of versene, washed three times and made into a working suspension in 0.85% NaCl, corresponding in density to a bacterial standard ($2 \cdot 10^9$ bacterial cells/ml).

The preparation was diluted as follows: to 3 drops of a corresponding dilution of the preparation we added 3 drops of the cell suspension, and the mixture was shaken. The results were read as with erythrocytes.

Agglutination was registered according to a four-grade system, depending on the formation of agglutinates as compared with the control. In all cases the control was a mixture of the corresponding suspensions with an equal volume of normal saline.

In the study of agglutinating properties of P-PHA toward bacteria we have used the same method as with cell material.

EXPERIMENTAL RESULTS

As seen from the table, the preparation studied agglutinated the different types of human erythrocytes, as well as those of the mouse, rooster and frog. The agglutinating properties of the preparation, therefore, are different toward the erythrocytes of different species of animals and of man. Thus mouse erythrocytes were agglutinated up to a dilution of 1:256, those of frog, up to 1:8. The agglutinating properties of the preparation were raised after refrigeration at 4°C.

It will be seen from the table that P-PHA agglutinated in vitro human cells (amnion, skin epithelium, fibroblasts), normal, as well as malignant (cancers of stomach, uterus and larynx), as well as cells of Ehrlich's mouse adenocarcinoma and rat ascitic ovarial cancer.

The agglutinating properties of the preparation were relatively weakly defined against human uterine cancer cells, laryngeal cancer cells and skin epithelium (titers 1:4, 1:8, 1:16). The agglutinating properties were comparatively well defined against human amnion cells and fibroblasts, and against ascitic cancer cells of mice and rats.

The best defined were the agglutinating properties against human stomach cancer cells; this reaction was characterized not only by a high titer, but also by its intensity, as the cells were agglutinated very densely. On the basis of our results we cannot conclude that this is a specific reaction, but this fact is worthy of note.

The table shows that P-PHA had no agglutinating properties against the bacteria studied. Phytohemagglutinins are antibodies of plant origin, and consequently they are obviously inactive toward bacteria.

The results of the study of the protein component of phytohemagglutinin of P. vulgaris, Sax variety, show its high agglutinating property toward erythrocytes of man and different animals and toward different cells of healthy and cancerous human tissues as well as toward ascitic cancer cells of mice and rats.

Our observations have shown a definite non-specificity in the action of this preparation. However there was a difference in titers and in the intensity of the agglutinating reaction against erythrocytes of different origins, and especially against different strains of healthy and cancerous human cells.

SUMMARY

A study was made of agglutinating properties of phytohemagglutinin obtained from the Sax variety of Phaseolus vulgaris (according to Rigas and Osgood's method) in the form of a protein. Agglutinating properties of phytohemagglutinin were tested against human (A, B, O), rabbit, rat, rooster and frog erythrocytes; single-layered human tissue cultures – normal cutaneous epithelium (strain No. 580), fibroblasts (strain No. 558), amnion (strain A-1); malignant cells of multilayered human tissue cultures – stomach cancer (strain Cave), cancer of the uterus (strain HeLa), cancer of the larynx (strain HEp-2); against malignant cells of experimental mouse cancer (Ehrlich's strain), cancer of the rat ovary (strain OR). Agglutination properties were also tested against an intestinal group of bacteria: Bacterium coli (strains C-85 and K-12) and Bacterium prodigiosum (strain No. 10).

As shown, phytohemagglutinins obtained from the Sax variety of Phaseolus vulgaris possessed high hemagglutinating properties. They were especially marked against mouse erythrocytes and comparatively weak against those of a frog. The preparation also possessed cytoagglutinating properties against all the cellular strains investigated. They were especially well pronounced in the instance of stomach cancer cells and least pronounced in that of uterus cancer cells, those of the larynx and cutaneous epithelium cancer. Phytohemagglutinin did not agglutinate the strains of bacteria examined. Agglutinating properties of the preparation are intensified after keeping it in a refrigerator (4°C).

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