IMMUNOBIOLOGICAL AND ELECTROPHORETIC INVESTIGATIONS ON PHYTOHEMAGGLUTININS IN THE SEEDS OF SOME BULGARIAN PLANT SORTS

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In the past two decades the scientific interest in antibodies of plant origin was substantially increased. This is due to the discovering of a vast number of specific phytohemagglutinins (3, 4, 9) as well as to other interesting biological properties of the same, namely their capacity of stimulating the mitotic activity of certain cells (1, 7, 11), of inhibiting the action of certain enzymes (10), of arresting the development of some tumourous cells in vitro etc. (5, 6).

Moreover, plant antibodies represent very suitable samples for carrying out a thorough study on immunological, biochemical and biophysical properties of the immune bodies in general.

In the present we report the results of our investigation on certain immunological and biochemical properties of phytohemagglutinins, extracted from the seeds of the sorts Phaseolus vulgaris (beans sort 4784, 4579, 4540), Soja hispida (soya bean sort Adams, soya sort "granular"), Pisum sativum (peas sort Ramonski and peas Peremiden), Lathirus aphaca (hatchet Taldjer).

Method and background of experiments. For the obtaining of phytohemagglutinins from the seeds of the plant sorts under investigation was employed the Rigas-Osgood's method (8) for receiving pure protein. The phytohemagglutininis thus obtained were studied in the following aspects:

1) Their agglutination properties were investigated to erythrocytes of human groups (A Rh^+, A Rh^-, B Rh^+, 0 Rh^+), mouse, rooster and frog. Titration was carried out with equal volumes of different, increasing in arythmetic progression dilutions of PHA and 2 per cent erythrocyte suspension. The agglutination was recorded aiter 45-minute refrigeration at +4 degrees C.

2) Investigations on the character, number and quantitative relations of protein fractions by means of paper electrophoresis. The latter was carried out under the following conditions: paper Watman I was used, tension of direct current fed to the pool 220-240 V and duration of electrophoresis — 6 hours. Veronal-acetate buffer solution was used with pH=8,9. Staining with Bromphenolblau according to routine methods. With each process a parallel sample was set with fresh normal human serum.

The quantitative determination of the fractions, obtained with the electrophoresis, was carried out according to the conventional method, employing the Pulfrich photometer and photometration with filters 61 and

57. On the basis of extinction values obtained, we determined the percentage relationship of the various fractions in the protein preparations under investigation.

In all the studies just described equal initial concentrations of the preparations were used — 3 gr/per cent, dissolved in 0.85 per cent sterile saline solution.

3) Studies were performed on the agglutination properties of the single, obtained at electrophoresis, fractions of PHA. For this purpose two samples were parallely set during electrophoresis, one of which was fixed and stained, and according to it in the second sample, the areas were cut out of the various fractions and extracted with saline solution. Ten drops saline solution were employed for the extraction of every single fraction, after which the eluates underwent centrifugation and were pipetted.

The agglutination properties of the eluates obtained from electrophoretic fractions of the studied preparations were verified with respect to human erythrocytes (A^+, B^+, 0^+ group) and to erythrocytes of mouse, rooster and frog.

Thus we have investigated the eluates of the PHA electrophoretic fractions of the three sorts Phaseolus vulgaris, one sort Soja Rispida, one sort Ramonski of Pisum sativum and one sort Taldjer hatchet Lathirus aphaca.

Results and analysis

The results of the investigations carried out in accordance to the tasks put before us are summarized in tables 1, 2, 3, 4 and 5.

In table 1, in which are presented the results of the studies on the agglutination properties of PHA obtained from the three Phaseolus vulgaris sorts, the strongly pronounced agglutination properties of the same are proved to all the erythrocytes investigated.

Yet, the analysis of agglutination titres showed that this capacity is manifested with different dilutions in relation to the erythrocytes used, according to their species and groups, which is indicative for the presence of a relative specifity in the PHA under study.

Furthermore, we were impressed by the big differences in agglutination titres toward the same type erythrocytes in the PHA relations from the three different beans sorts. This phenomenon was elucidated in the light of the results of investigation on the character of electrophoretic fractions of PHA from beans, their agglutination properties and quantitative relationships.

Table 4 shows that with paper electrophoresis the PHA obtained from the three sorts beans are divided in two fractions: one with the motility of the γ-globulins of human serum, and the other — with mobility similar to that of α_2-globulins. It is estbalished also, that the first fraction constitutes the greater part (73%, 80%, 83%) of the total quantity isolated globulins. In the course of studying the agglutination property of the eluates in these fractions we found out, as illustrated in table 5, that only the former fractions (with the motility of the human γ-globulins) possess such a properties.

Table 1

Sort 4540

Type of Er	PHYTOHEMAGGLUTININ DILUTIONS															
	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	Control	Titre
A+	++++	++++	++++	++++	++	++	+	+	+	+	−	−	−	−	−	1: 512
A−	++++	++++	++++	++++	+++	+++	+++	+++	++	++	−	−	−	−	−	1: 512
B+	++++	++++	+++	+++	++	++	++	+	−	−	−	−	−	−	−	1: 128
0+	++++	++++	++++	+++	++	++	+	±	±	−	−	−	−	−	−	1: 64
mouse	++++	++++	++++	++	++	++	++	++	++	++	+	+	−	−	−	1:2048
rooster	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	−	−	−	−	1:1024
frog	++	+	+	+	±	±	−	−	−	−	−	−	−	−	−	1: 8

Sort 4579

A+	++++	++++	+++	++	+	+	+	+	+	+	+	−	−	−	−	1:1024
A−	++++	++++	++++	++	++	++	++	+	+	+	+	+	−	−	−	1:2048
B+	++++	++++	++++	++	+	+	+	+	+	+	−	−	−	−	−	1: 512
0+	+++	+++	++	++	+	+	+	+	+	+	+	−	−	−	−	1:1024
mouse	+++	+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	−	1:8192
rooster	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	++	−	−	1:4096
frog	+++	+	+	+	+	+	−	−	−	−	−	−	−	−	−	1: 32

Sort 4784

A+	++	++	++	+	+	[illegible]	++	±	−	−	−	−	−	−	−	1: 64
A−	++++	++++	+++	+++	++	++	++	+	−	−	−	−	−	−	−	1: 128
B+	++++	++++	++	++	++	++	+	−	−	−	−	−	−	−	−	1: 64
0+	++++	++++	++	++	+	±	−	−	−	−	−	−	−	−	−	1: 16
mouse	++++	++++	+++	++	++	++	++	++	++	++	+	−	−	−	−	1:1024
rooster	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	−	−	−	−	−	1: 512
frog	++	+	+	+	+	+	−	−	−	−	−	−	−	−	−	1: 32

In the table the results are presented of investigations on the agglutination properties of PHA from seeds of the three sorts Phaseolus vulgaris. Concentration of PHA 3 gr/%.

Table 2

Sort peremiden

Type of Er	Phytohemagglutinin dilutions														
	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	Control	Titre
A^+	+++	++	++	–	–	–	–	–	–	–	–	–	–	–	1: 4
A–	+++	++	++	–	–	–	–	–	–	–	–	–	–	–	1: 4
B^+	+++	+++	++	–	–	–	–	–	–	–	–	–	–	–	1: 4
0^+	+++	+++	+++	++	–	–	–	–	–	–	–	–	–	–	1: 8
mouse	–	+	+	+	+±	+±	+	–	–	–	–	–	–	–	1:64
rooster	++++	++++	++++	+++	–	–	–	–	–	–	–	–	–	–	1: 8
og	+	±	–	–	–	–	–	–	–	–	–	–	–	–	1: 1

Sort Ramonski

A^+	++++	+++	+++	++	+	+	±	–	–	–	–	–	–	–	1: 32
A–	++++	+++	++	++	++	+	+	–	–	–	–	–	–	–	1: 64
B^+	++++	+++	+++	+++	+	+	–	–	–	–	–	–	–	–	1: 32
0^+	++	++	+	+	–	–	–	–	–	–	–	–	–	–	1: 8
mouse	++	++	++	++	++	++	++	++	++	–	–	–	–	–	1:256
rooster	++++	++++	++	–	–	–	–	–	–	–	–	–	–	–	1: 4
frog	++	+	+	+	–	–	–	–	–	–	–	–	–	–	1: 8

In the table the results are illustrated of investigations of the agglutination properties of PHA from two sorts Pisum sativum, at concentration of the preparation 3 g1/%.

Table 3

Sort Adams

Type of Er	Phytohemagglutinin dilutions															
A+	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	Control	Titre
A−	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	+	−	−	−	−	1:1024
B+	++++	++++	++++	++++	++++	++	+	−	−	−	−	−	−	−	−	1: 64
0+	++++	++++	++++	++++	++++	++++	+	±	−	−	−	−	−	−	−	1: 64
mouse	+++	+++	+++	+++	+++	+++	++	+	±	−	−	−	−	−	−	1: 128
rooster	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	−	1: 4
frog	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	−	1: 4
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0

Sort yellow granular

A+	++++	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	1:8
A−	++++	++++	+++	+	±	−	−	−	−	−	−	−	−	−	−	1:8
B+	++++	++++	+++	++	−	−	−	−	−	−	−	−	−	−	−	1:8
0+	++++	++++	+++	+	−	−	−	−	−	−	−	−	−	−	−	1:8
mouse	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
rooster	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
frog	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0

Hatchet

A+	++++	++++	+++	++	++	+	−	−	−	−	−	−	−	−	−	1:32
A−	++++	++++	+++	+++	+++	++	−	−	−	−	−	−	−	−	−	1:32
B+	++++	++++	++++	++++	++++	++++	−	−	−	−	−	−	−	−	−	1:32
0+	+++	++	++	++	+	−	−	−	−	−	−	−	−	−	−	1:16
mouse	+++	++	++	++	++	++	++	±	−	−	−	−	−	−	−	1:64
rooster	++++	++++	++++	++++	+	−	−	−	−	−	−	−	−	−	−	1:16
frog	++++	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	1: 8

In the table the results are presented of investigations on the agglutination properties of PHA from the seeds of two sorts Soja hispida and one sort Lathirus aphaca. Concentration of PHA 3 gr/%.

The parallelism established between the percentage content of the first fraction and the height of agglutination titres also supports the inference to the effect that capacity for agglutination is possessed only by the

Table

TYPE SORT		α	β	α_2	α_1	Alb
Control human serum		0,19(17,92%)	0,17(16,04%)	0,10(9,53%)	0,13(12,26%)	0,47(44,34%)
Phaseolus vulgaris	4540	0,51(80,95%)	—	0,12(19,05%)	—	—
	4579	0,15(83,33%)	—	0,03(16,66%)	—	—
	4784	0,22(73,33%)	—	0,08(26,66%)	—	—
Soja hispida	soya granular	0,16(37,21%)	0,25(58,16%)	0,02(4,65%)		—
	soya Adams	0,27(72,97%)		0,10(27,02%)		—
Pisum sativum	Peas Ramonski	0,11(21,57%)	0,19(37,25%)	0,12(23,53%)	0,07(13,72%)	0,02 (3,92%)
	Peas Peremiden	0,11(18,64%)	0,17(28,81%)	0,13(22,03%)	0,14(23,73%)	0,04 (6,78%)
Lathirus aphaca	Hatchet Taldjer	—	0,05(71,43%)	0.02(28,57%)	—	—

The table illustrates the extinction values and percentage relationships of the established by means of paper electrophoresis fractions in the Phytohemagglutinins of Phaseolus vulgaris, Soja hispida, Pisum sativum and Lathirus aphaca.

globulins of Ph. vulgaris, which correspond in mobility to the human γ-globulins. This finding explains the differences manifested in the power of agglutination properties from the three beans sorts, as to the higher percentage content of the first fraction corresponds a more strongly pronounced agglutination property.

The comparison of PHA from beans 4784, 4540 and 4579 with the respective percentage content (73%, 80% and 83%) of the first fraction accounts for a different agglutination capacity. For example, their titres to human A^+ erythrocytes are 1:64, 1:512 and 1:1024 respectively. The picture is similar as regards the remaining erythrocytes. Especially strongly manifested are the agglutination properties of PHA from beans to mouse and rooster erythrocytes, which property is manifested not only with respect to major dilutions, in which it is positive, but with respect to intensity as well. The weakest in so far strength and intensity is concerned, is the agglutination capacity of the PHA to frog erythrocytes, whereas within the limits of human species — to the $O+$ group erythrocytes.

These results are in compliance with the data of our prior investigations on agglutination properties of PHA fom Ph. vulgaris, sort Sax (2).

The analysis of table 2, in which the results are presented of exploring the agglutination properties of two sorts peas, shows a weaker manifestation as compared to that of beans.

Besides that, differences exist in the attitude towards some of the erythrocytes used. Quite typical are the comparatively more weakly manifested agglutination properties to erythrocytes of rooster in comparison to those of the beans PHA. On the whole, the agglutination power of the Peas PHA is weaker.

Table 5

Type of PHA	To the erythrocytes of					
Phaseolus vulgaris	A^+	B^+	0^+	mouse	rooster	frog
sort 4540	++++	++++	++++	+ +++	++++	+
	–	–	–	–	–	–
sort 4579	++++	++++	++++	++++	++++	+
sort 4784	–	–	–	–	–	–
Soja hispida	++++	++++	++++	++++	++++	+
sort	–	–	–	–	–	–
granular	++++	+++	+++	–	–	
	–	–	–	–	–	–
Pisum sativum	–	–	–	–	–	–
sort						
Ramonski	++++	++++	++	+++	–	+
	–	–	–	–	–	–
Lathirus aphaca	–	–	–	–	–	–
sort	–	–	–	–	–	–
Taldjer	–	–	–	–	–	–

Results of investigations on agglutination properties of eluates from PHA electrophoretic fractions of Phaseolus vulgaris, Soja hispida, Pisum sativum, Lathirus aphaca.

Here too, the results of electrophoretic investigations indicate that the causes are of the nature already described.

As illustrated in Fig. 1, the electrophoretic study of Peas PHA reveals five fractions which do not fully correspond, but are only similar in their motility to the electrophoretic fractions $\gamma, \beta, \alpha_2, \alpha_1$, and albumins of human blood serum.

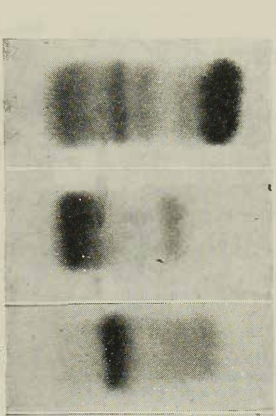

Fig. 1: Electrophoregram of phytohemagglutinin (PHA) from Phaseolus vulgaris, Soja hispida, Pisum sativum, isolated according to the method of Rigas and Osgood.

I — conrtol human blood serum;
II — Phaseolus vulgaris (beans) phytohemagglutinin;
III — Soja hispida (soya) phytohemagglutinin;
IV — Pisum sativum (peas) phytohemagglutinin,
(veronal — acetate buffer ph 8, 9, duration 6 hours).

The percentage relationship in these fractions, as shown in table 4, is different for the two sorts peas, the highest being in both instances that of tne second fraction (β).

On the basis of investigations on agglutination properties of these fractions' eluates, the results of which are given in table 5, the conclusion is reached that agglutination capacity is inherent to the $\gamma+\beta$ fractions'eluates, and is absent in the eluates from the α_2- and α_1-fractions. The differences in agglutination titres between PHA of the two sorts peas could be explained with the quantitative interrelations between these fractions. The high-percentage content of the $\gamma+\beta$-fractions in the Ramonski sort (58,82%) is in accordance to the more strongly pronounced agglutination capacity.

Against the background of studying the agglutination properties of the PHA, isolated from soja, sort Adams and soja, sort yellow granular, the results of which are presented in table 3, the agglutination capacities were established to human, mouse and rooster erythrocytes with the Adams sort, whereas, with yellow granular soja — only with respect to human erythrocytes A^+ A^-, B^+, 0_+.

The data here submitted are indicative for a markedly manifested relative specifity in PHA obtained from the soja seeds.

Of special interest is the elucidation of the differences in agglutination properties of the soja, beans and peas PHA, in the light of data furnished by the electrophoretic analysis.

It is clearly evident in Fig. 1 and table 4 that in the PHA, isolated from the seeds of both sorts soja by means of paper electrophoresis, four fractions are disclosed, of which the first two correspond, as far as motili-

ty is concerned, to the γ and β globulins of the control human serum, whereas the second two fractions are not sufficiently well differentiated and are similar in their motility to the α_2 and α_1-globulins of the control human serum.

As demonstrated in tables 1 and 4, the highest is the percentage content in the second electrophoretic fraction, which is endowed with the motility of the β-globulins of the control human serum.

The great differences in the quantitative interrelations between γ+β-fractions and $\alpha_2+\alpha_1$-fractions in both sorts soja were very impressive. 95,37% γ+β-globulin fractions were observed with the yellow granular soja, whereas with the sort Adams soja — 72,57%. Such quantitative differences compared to the agglutination properties of the total PHA, obtained of both sorts, do not warrant the inference that agglutination properties are characteristic for the first and second electrophoretic fraction. The results of the investigations on the agglutination properties of eluates from the soja yellow, granular PHA electrophoretic fractions, presented in table 5, show that a capacity for agglutination is possessed by the eluate obtained from γ+β-fraction, whereas in the α_2 and α_1-fractions it is absent. In addition, this capacity is manifested only towards human erythrocytes, and not to those of mouse, rooster and frog.

The following fact is worth of special mentioning: the narrowing of the spectrum of soja PHA agglutination capacity is in accordance to the higher percentage content of β-globulin fractions, whereas the lower — to the $\alpha_2+\alpha_1$-fractions. In the yellow, granular sort soja, which has 58,16% β-fraction and only 4,65% $\alpha_2+\alpha_1$-fractions, agglutination capacity is manifested only to human erythrocytes, whereas with the soja sort Adams in which the quantity of the γ+β-fractions is 72,97%, and that of the $\alpha_2+\alpha_1$-fractions 27,02%, the agglutination capacity is manifested in a higher dilution and in a wider spectrum.

The data at our disposal at the present moment are not sufficient for the definitive clarification of this phenomenon, but the regularities established justify the hypothetic presumption that the rest of the fractions, which alone do not manifest proper agglutination properties, exert certain influence on the properties of the total PHA in compliance to the species and quantitative relationships.

In table 3 the results are also presented from investigating the agglutination properties of PHA, obtained from the seeds of hatchet sort Taljersko (Lathirus aphaca). The latter has a relatively well pronounced agglutination capacity and in this respect it resembles the PHA of Phaseolus vulgaris. The electrophoretic fractions which were obtained from the PHA, isolated from the hatchet are two: one with the motility of β-, and the other with the motility of α_2-globulins of the control human blood serum. In the eluates thus obtained, agglutination properties were not found to the erythrocytes used, as illustrated in table 5. This is an interesting fact and could be explained with the presumption that it is due to a rather weak concentration of the respective eluate fractions, or with the joint action of both fractions. Anyway, the elucidation of this question is the scope of further investigations.

Conclusions

The obtained with the Rigas and Osgood method protein fractions from the seeds of various sorts Phaseolus vulgaris, Soja hispida, Pisum sativum, Lathirus aphaca represent substances of the globulin type. By means of electrophoresis two fractions are established in the PHA isolated from Ph. vulgaris, which correspond in their motility to the γ- and β- globulins of the control human serum. In Soja hispida we discovered four electrophoretic fractions, corresponding in motility to the γ β-, α_2-, α_1- globulins of the control human serum. With Pisum sativum we established 5 fractions with motility resembling the γ-, β-, α_2-, α_1 globulins and the albumins of the human blood serum, whereas in Lathirus aphaca — two fractions with motility of the β- and α_2-globulins of the same.

The finding that there exist differences not only in the number and type of electrophoretic fractions in the separate PHA types, but in the percentage relationships as well is very characteristic. The variations manifested within the limits of the species refer merely to the percentage relationships of electrophoretic fractions and not to their number or nature.

The PHA obtained from the four species posses the property to agglutinate human erythrocytes from the gr. A^+, A^-, B^+ and 0^+, and those of mouse, rooster and frog. This property is differently manifested in the four species, as in Soja hispida yellow granular sort, agglutination is absent with respect to rooster and frog erythrocytes, whereas in the Adams sort — with respect to frog erythrocytes.

The results obtained are indicative for a markedly manifested relative specificity in the agglutination property of the PHA under study.

We found out also that agglutination capacity of the isolated type is possessed only by the γ- and β -fractions of the PHA explored.

These data, as well as the differently manifested agglutination properties for each species, combined with the characteristic electrophoretic data warrant the inference, that for the various agglutination capacities thus manifested of PHA from the species cited, are essential not only the fractions, capable of independent erythrocytic agglutination, but the remaining fractions as well, according to their nature and quantitative relationships. The latter may have an effect on the nature or strength of the agglutination capacity, inhibiting or stimulating it in one or other respect. The differences thus established in the number, character and percentage relationships of the fractions in the various PHA, very probably, account for the characteristic agglutination properties in them. This suggestion of ours, based on the results obtained and presented in the present paper, will serve as operative hypothesis in our future investigations in this field.

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ИММУНОБИОЛОГИЧЕСКИЕ И ЭЛЕКТРОФОРЕТИЧЕСКИЕ ИЗУЧЕНИЯ ФИТОГЕМ-АГГЛЮТИНИНОВ В СЕМЕНАХ НЕКОТОРЫХ НАШИХ РАСТИТЕЛЬНЫХ СОРТОВ

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РЕЗЮМЕ

Сообщаются результаты изучений над агглютинационными свойствама и электрофоретическими фракциями фитогемагглютининов разных сортов и видов Phaseolus vulgaris, Pisum sativum, Soja hispida, Lathirus aphaca.

Фитогемагглютинины получены в виде чистого протеина, по методу Rigas и Osgood.

Установлено, что разные фитогемагглютинины имеют характерную для данного вида относительную агглютинационную специфичность в отношении эритроцитов человека, мыши, петуха и лягушки.

Посредством електрофореза на бумаге были установлены типичные фракции для фитогемагглютининов каждого вида, которые идентифицируются по подвижности с электрофоретическими фракциями глобулинов сыворотки крови человека.

Было установлено, что только фракции, которые соответствуют по их подвижности гама- и бета-сывороточным глобулинам человека имеют агглютинационные свойства. В разных сортах данного вида были установлены только количественные, но не и качественные различия в белковых фракциях, которые дают отражение на титровые стоимости агглютинации, но не и на ее специфичность.