Chemistry of Natural Compounds, Vol. 37, No. 2, 2001

PHYTOHEMAGGLUTININS FROM *Phaseolus vulgaris* L. AND *Pisum sativum* SEEDS

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UDC 581.19

The isolation and purification of phytohemagglutinins from Phaseolus vulgaris *and* Pisum sativum *seeds are reported.*

Key words: phytohemagglutinin, hemagglutinating and mytogenic activity.

Phytohemagglutinins (PHA), or lectins, are studied owing to their specificity for blood-type antigens. Furthermore, they are widely used to stimulate mitosis in tissue culture [1], to stimulate biosynthesis [2], to discover late rare erythrocytotic antigens [3, 4], and to separate formal blood elements [5]. PHA belong chemically to glycoproteins with molecular weight 130,000. They contain 10-15% covalently bound carbohydrate.

The goal of the present work was to isolate and purify the PHA protein fractions from *Phaseolus vulgaris* (two varieties, white and colored) and *Pisum sativum* and to determine their hemagglutinating and mitogenic activities.

Seeds of white and colored beans and peas were ground and extracted with phosphate buffer (0.1 M, pH 7.4) containing NaCl (0.9%) in order to isolate PHA. Then fractionation was performed using $(NH_4)_2SO_4$ (Table 1).

The protein fractions obtained at 20-35% $(NH_4)_2SO_4$ saturation exhibited a high anti-A PHA titer. Their activities were significantly higher than those of the starting extracts. All active fractions were separated by chromatography. Pure protein fractions were isolated on a DEAE-Sephadex A-50 column using literature methods for separation of serum proteins [5].

Figure 1 illustrates the fractionation of white-bean PHA preparation. It can be seen that the fraction with anti-A activity is eluted by phosphate buffer (0.05 M, pH 7.4). It should be noted that the gamma-globulin fraction is isolated from human blood serum under these same conditions [6]. Therefore, fraction F-1, which possesses phytohemagglutinating activity, has the same sorptivity on ion-exchange gel and elutes analogously to human blood-serum gamma-globulin. The isolated F-1 also had mitogenic properties. Fractions F-1 from colored-bean and peas had analogous properties. The mitogenic properties were determined using human blood leucocytes to produce dividing cells [7]. Lymphocytes experiencing blast transformation undergo active multiplication in blood culture with PHA mitosis stimulators. The mitogenic properties of white-bean PHA were determined by the literature method [8].

Table 2 contains the results. The normal karyotype 46XV was established in all preparations. Mitogenic activity was also noted in colored-bean and pea PHA in these preparations.

Thus, PHA that are used in immunochemical and cytogenetic analyses are isolated and partially purified from extracts of seeds from *Phaseolus vulgaris* (two varieties) and *Pisum sativum*.

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0009-3130/01/3702-0185$25.00

TABLE 1. Fractionation of Proteins by Ammonium Sulfate

Extract fraction	Total protein content, mg	Anti-A PHA titer
Colored beans	1500	$1:2^{15}$
I	86	$1:2^{12}$
II	126	$1:2^{12}$
III	350	$1:2^{6}$
IV	300	$1:2^{9}$
White beans	1400	$1:2^{15}$
I	80	$1:2^{12}$
II	120	$1:2^{12}$
III	375	$1:2^{6}$
IV	290	$1:2^{9}$
Peas	1430	$1:2^{15}$
I	75	$1:2^{12}$
II	128	$1:2^{12}$
III	390	$1:2^{6}$
IV	300	$1:2^{7}$

Precipitation (%): 0-20 (I), 20-35 (II), 35-65 (III), 65-100 (IV).

TABLE 2. Cytogenetic Analysis of PHA Effect on Mitosis

Glass slide No.	Metaphase plates	
	exptl.	control
1	12	18
2	17	29
3	10	31
4	15	16
5	9	21
6	18	13
7	26	11
8	11	19
9	19	25
10	29	23
11	29	26
12	20	19

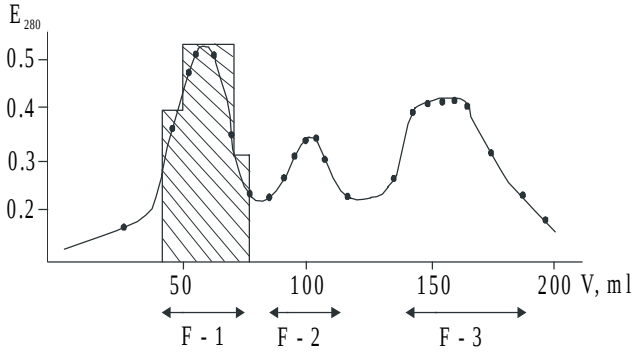

Fig. 1. Fractionation of phytohemagglutinating fraction of white bean on a DEAE-Sephadex A-50 column. The anti-A PHA titer is hatched. Fractions eluted by phosphate buffers: 0.05 M, pH 7.4 (F-1); 0.1 M (F-2); 0.2 M (F-3).

EXPERIMENTAL

Bean (white and colored) and pea seeds were grown in Uzbekistan, ground into powder (10 g each), and extracted with phosphate buffer containing NaCl (0.9%) in a 1:10 ratio for 3 h. The extracts were centrifuged for 15 min at 3000 rpm.

Fractionation using $(NH_4)_2SO_4$ was performed as follows: Supernatant (100 mL) was treated with $(NH_4)_2SO_4$ (10.88 g). The resulting precipitate was separated by centrifugation. The supernatant was treated again with $(NH_4)_2SO_4$ (20-35%, calculated at 8 g per 100 g of solution). The next precipitations used 35-65% (20 g per 100 g solution) and 65-100% saturation (28 g per 100 g solution).

The PHA activity was determined by titration of preparation with a 20% suspension of human erythrocytes. Lectin titration was performed in normal saline (9% NaCl) on a plate for 10 min by double dilution. The activity of the preparation was judged from the greatest dilution at which erythrocytes agglutinate in 10 min.

Ion-exchange chromatography on DEAE Sephadex A-50 was carried out in a column (2×25 cm) in phosphate buffer (pH 7.4) with gradual addition to the starting buffer of NaCl (from 0.1 to 0.2 N).

Samples (5 mL) were collected. The absorbance at 280 nm was determined, and protein was determined using the Lowry method [9]. Elution curves were constructed.

Mitogenic activity was determined in six vials containing blood (1 mL) by adding PHA solution (eight drops, 1 mg/mL). The control was PHA (Difco). The vials were placed in a thermostat (37°C) for 72 h. Colchicine was added on the third day to stop dividing cells in metaphase. After treatment, chromosome preparations were prepared on glass slides and were colored with Giemsa dye. Chromosome condensation and morphology were studied using a Lics Galen III microscope. The normal karyotype 46XV was found in all preparations.

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