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Isolation, Purification and Characterization of Phytohemagglutinating Proteins from Korean Natural Products

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Abstract□Seeds or beans of 55 plants belonging to 31 families were screened by using several different types of red blood cells to find new lectins. In this paper, white kidney bean (*Phaseolus vulgaris* C.) was chosen to study biochemical properties of hemagglutinating proteins(lectins). An anion exchanger, DEAE Sephadex A-50, and polyacrylamide disc gel electrophoresis were main techniques used. From three main fractions eluted by stepwise NaCl gradient in 25mM Tris-HCl buffer on DEAE Sephadex column, principal lectin was identified.

Keywords □ Haemagglutinating proteins - Particular compounds such as enzymes, antibodies and lectins -Phytohemagglutinin-Erythroagglutinin- Erythroagglutinating activity-Serial two-fold dilutions method on a microtiter system-White kidney beans-$(NH_4)_2SO_4$ precipitate-DEAE Sephadex A-50 column-A_{280}-Stepwise increasing NaCl gradient-Polyacrylamide disc gel electrophoresis.

A living organism, in nature, contains a large variety of proteins that possess the ability to bind specifically different compounds such as enzymes, antibodies and lectins. These three are very similar in a certain biochemical point of view, but they are very different with one another. [1,2,3] A very particular constituent, usually found in plants, that possesses the remarkable ability to agglutinate erythrocytes and other types of cells is known as phytohemagglutinin, but relatively recently named as lectin by Boyd[4,5]. Lectin has several remarkable biological or biochemical activities such as the ability to bind sugars, to agglutinate erythrocytes, to agglutinate leucocytes, to stimulate lymphocytes, the anti-tumor activity and the toxicity to living organisms[6,7,8].

The object of this paper is to find new lectins from Korean natural products and to establish a new method for isolation, purification and characterization of new lectins.

MATERIALS AND METHODS

Screen Test

More than 50 kinds of collected seeds and beans (cultivated or wild) were screened to find whether they contain phytohemagglutinin. Crude extracts were made from 0.15 M saline solution. Blood bank blood was purchased and washed in 0.15 M saline and the erythrocytes were separated by centrifugation. The cells were then suspended as three per cent and were used within two hours of preparation for the erythroagglutinating activity test. This test was carried out by the serial two-fold dilutions method on a

Table I: Blood group specificity of crude plant lectins

Plant origins	Human Blood[a)]			Rabbit	Specif[b)]. Signif.
	A	O	AB		
Amarantaceae					
Celosia cristata	H	H	H	O	NS
Apiaceae					
Foeniculum vulgare	0	0	0	512	RB
Aquifoliaceae					
Euonymus japonica	1024	64	1024	4096	NBS
Araceae					
Pinellia ternata	0	0	2	16	RB
Bignoniaceae					
Catalpa ovata	0	0	0	64	RB
Brassicaceae					
Brassica juncea	O	H	O	512	RB
Buxaceae					
Buxus microphylla	O	O	O	O	NS
Campanulaceae					
Platycodon grandiflorum	H	H	H	H	NS
Compositae					
Bidens parviflora	O	O	O	4096	RB
Helianthus annus	O	O	2	2	NS
Convoivulaceae					
Pharbitis nil	H	O	H	H	NS
Cornaceae					
Cornus officinalis	16	32	16	4096	NBS
Cruciferae					
Raphanus sativus	O	O	O	128	RB
Cucurbitaceae					
Cucumis melo	O	O	O	O	NS
Citrullus vulgaris	O	O	O	256	RB
Cupuriferae					
Castanea crenata	2	O	64	2	AB
Ginkgoaceae					
Ginkgo biloba	O	O	O	2048	RB
Gramineae					
Hordeum vulgare	O	O	O	O	NS
Coix mayuen	O	O	O	O	NS
Juglandaceae					
Juglans sinensis	8	2	4	4096	RB
Leguminosae					
Cassia tora	O	O	O	O	NS
Cercis chinensis	256	32	1024	4096	NBS
Glycine may A	4	4096	4	4096	O,RB
Glycine may B	O	O	O	128	RB
Glycine may C	4	O	8	4096	RB

Glycine may D	8	O	8	4096	RB
Glycine may E	O	O	O	4096	RB
Glycine may F	O	O	O	4096	RB
Phaseolus angularis	O	O	O	64	RB
P. radiatus	O	O	O	4096	RB
P. vidissimus	O	O	O	32	RB
P. vulgaris A(red)	4096	4096	256	2048	NBS
P. vulgaris B(yellow)	512	4096	4096	4096	NBS
P. vulgaris C(white)	1024	4096	1024	4096	NBS
Pisum sativum	16	8	16	512	NBS
Vicia faba	16	O	O	1024	A,RB
Liliaceae					
Allium fistulosum	O	O	O	O	NS
Allium odoratum	O	4	O	O	NS
Liriope muscari	O	O	O	4096	RB
Malvaceae					
Hibiscus manihot	O	H	O	4096	RB
Moraceae					
Ficus carica	H	8	128	H	AB
Nymphaeaceae					
Nelumbo nucifera	O	4	O	O	NS
Pinaceae					
Thuja (Biota) orientalis	O	O	O	4096	RB
Plantaginaceae					
Plantago asiatica	O	O	O	2048	RB
Punicaceae					
Punica granatum	H	H	8	2	NS
Rhamnaceae					
Zizyphus vulgaris	O	O	O	O	NS
Z. vulgaris L. *var. spinolus*	O	O	O	256	RB
Rosaceae					
Prunus armeniaca	O	O	O	2048	RB
Rosa centifolia	O	2	H	2048	RB
Rubiaceae					
Gardenia jasmenoides	H	8	8	O	NS
Rutaceae					
Xanthoxylum piperitum	O	O	O	128	RB
Solanaceae					
Capsicum annuum	H	H	H	2	NS
Lycium chinense	64	O	O	4096	A,RB
Vitaceae					
Vitis thunbergii	16	4	16	2048	NBS
Vitis vinifera	32	128	32	4096	NBS

a) H: haemolysis, numbers from O to 4096 indicates the activity of erythroagglutination.

b) Specif.: specificity to blood type. Signif.: significance as lectin resources, NS: no significance, NBS: no blood specificity, RB: rabbit blood specificity, O: o type specificity, A: A type specificity, AB: AB type specificity

a microtiter system(see below Hemagglutinin Assay).

Extraction

For the following further studies, we have choosen white kidney beans (*Phaseolus vulgaris, Leguminosae*). Twenty grams of Korean white kidney beans, purchased from grocers, were ground and extracted with 200 ml of 0.15 M NaCl for 24 hours at 4 C and centrifugated at 8,000 rpm for 10 min and the pellet was discarded. Ammonium sulfate was added to 40 % (226 mg/ml) over a 30 min period to the stirred crude extract and after overnight, the precipitate was removed by centrifugation and discarded. Additional ammonium sulfate was added to 90 % (335 mg/ml) as before and the resulting precipitate was collected by centrifugation at 8,000 rpm for 30 min.

Purification of Lectin through DEAE Sephadex A-50

The 40–90 % $(NH_4)_2 SO_4$ precipitate was dissolved in 50 ml of 0.15 M NaCl solution and thoroughly dialysed against the same solution for overnight and subsequent dialysis were performed against 25 mM Tris-HCl buffer (pH 7.2). The crude extracts were applied onto DEAE Sephadex A-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) column, which was pre-equilibrated with 25 mM Tris-HCl buffer. The column was washed with 25mM Tris-HCl buffer until the A_{280} of the effluent was below 0.05 and no hemagglutinating activity was observed, in the washing. Then the column was eluted subsequently using a stepwise increasing NaCl gradient from 0.05 M, 0.1 M and to 0.2 M in 25 mM Tris-HCl buffer.

Polyacrylamide Disc Gel Electrophoresis

The method of Jeune[3,4] was used, which involved electrophoresis in columns (5×70 mm) of polyacrylamide gel in Tris-Glycine buffer, pH 8.3 with bromphenol bule as a marker.

Hemagglutinin Assay

Lectin activity was measured by the ability to agglutinate human blood type A, AB, O and rabbit blood erythrocytes. Serial 2-fold dilutions of eluted proteins from DEAE Sephadex A-50 were incubated at room temperature with 50 μl of 3 % erythrocytes in 0.15 M NaCl. After 30 min, the microtiter plate was examined both macroscopically and microscopically.

RESULTS AND DISCUSSION

Lectin Resources

While testing crude extracts for erythroagglutinating activity for wild or cultivated beans and seeds of natural products, we detected variable hemagglutinating activity with human blood type A, AB, O and rabbit blood erythrocytes (Table I and Fig. 1). As summarized in Table I, the extracts from 55 different species of plants belonging to 31 families were compared for their erythroagglutinating activity with different blood groups. In major cases, rabbit blood showed particularly strong activity (37 species from 55). We also noticed that *Phaseolus* species had no blood type specificity to human

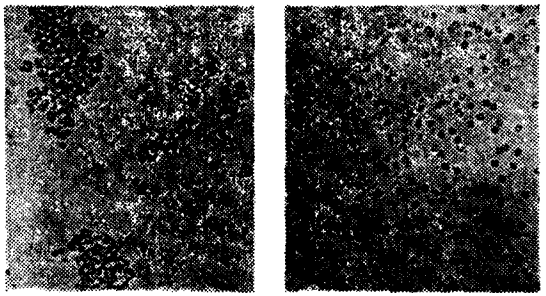

Fig. 1: Normal A-type human erythrocytes suspended in 0.15 M NaCl solution as 3 % (left) and agglutinated cells after incubation for 30 min with 25 μg of crude lectin extract from white kidney beans (right) (1×100)

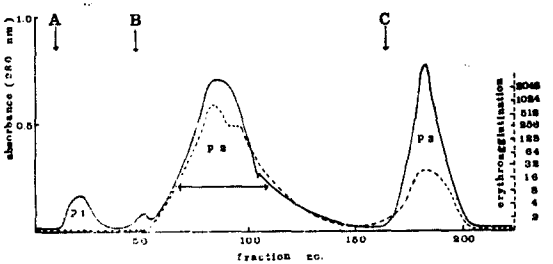

Fig. 2: Elution profiles of white kidney beans lectins on DEAE Sephadex A-50 column. absorbance,——; erythroagglutination, ……; fraction volume, 5 ml; A, 0.05M NaCl gradient in 25 mM Tris-HCl buffer; B, 0.1 M NaCl gradient in 25 mM Tris-HCl buffer; C, 0.2 M NaCl gradient in 25 mM Tris-HCl buffer.

blood. Relatively strong hemolysis was observed on several seeds.

Characterization of Principal Protein Constituent

The elution profiles of crude extracts on a DEAE Sephadex A-50 column are shown in Figure 2. Considerably large amounts of unfixed proteins were eluted while sample washing. But we obtained three peaks by stepwise NaCl gradient in 25 mM Tris-HCl buffer elution. Characterization of these three peaks are summarized in Table II. It is noted that the peak 2 (p2) has reasonable results not only by its volume, but also by its optical density and by its high activity of erythroagglutination. This fraction is worthwhile to carry out further purification and immunobiochemical studies and the authors intend to report results in due courses.

Polyacrylamide Disc Gel Electrophoresis

Results of electrophoretic studies are shown in Figure 3. More than seven bands can be read from crude extracts gels, shown at the

Table II: Characterization of three major proteins eluted from DEAE Sephadex A-50 column.

Peaks	A_{280}	Volume (ml)	Agglutinating activity
p 1	0.13	75	0
p 2	0.48	220	256
p 3	0.48	125	16

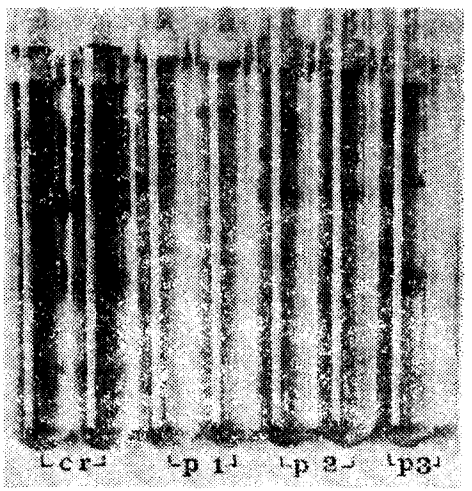

Fig. 3: Comparison of polyacrylamide disc gel electrophoretic patterns of crude extracts and pooled fractions after DEAE Sephadex A-50 column. Migration was from top to bottom. cr, crude extract; p 1, p 2 and p 3 corresponds to the pooled fractions of peaks 1, 2 and 3 eluted as in Fig. 2.

left, and cr. p 1, p 2 and p 3 corresponds to the peaks eluted by DEAE Sephadex A–50 column. According to this electrophoretic pattern, it is considered that the peak 2 is reasonably good for principal lectins from *Phaseolus vulgaris* C., white kidney beans.

CONCLUSION

We showed that a possibility of finding new lectins form Korean natural products is unlimitted. In the present report, we have screened 55 different kinds of beans or seeds of medicinal plants. It was shown that peak 2 of Figure 2 is a principal hemagglutinating protein(lectin) from white kidney beans. The peak 2 had acceptable criteria, e.g., optical density, yields as volume and agglutinating activity. Another purity criterion of the peak 2 which supports our conclusion as principal lectin is electrophoretic studies as in Figure 3.

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