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Blastogenic Property of Lectin from Semen Jequirity (*Abrus precatorius L.*)

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Abstract. Lectin was extracted from the seeds of *Abrus precatorius L.* by affinity chromatography and ion exchange column chromatography and the purified lectins ($AL\text{-}II_1$ and $AL\text{-}III_2$ fractions) which had high hemagglutinating activity were obtained. The hemagglutinating activity of the $AL\text{-}III_2$ fraction was higher than that of the $AL\text{-}II_1$ fraction. On the other hand, the toxicity of the $AL\text{-}III_2$ fraction was comparatively lower than that of the $AL\text{-}II_1$ fraction. These abrus lectins induced ^{3}H-TdR incorporation, an increase in Feulgen-DNA content and blastoid transformation of human blood lymphocytes in vitro. The maximal degrees of blastoid transformation were observed to be 4.5% in the $AL\text{-}II_1$ fraction and 6.4% in the $AL\text{-}III_2$ fraction. The blastoid cells induced by the abrus lectin resembled those of PHA culture in both size and staining characteristics. These results indicate that the abrus lectin has blastogenic property.

Key Words: abrus lectin, lymphocyte blastogenesis

Introduction

The seeds of *Abrus precatorius L.* are known to contain a high toxic protein, lectin or both(1) and their biological properties have been reported (2-4). There is, however, little information on blastogenic properties of the abrus lectin except that galactose binding abrus lectin stimulated DNA synthesis of human blood lymphocytes in vitro (5). Therefore, we tried to purify toxin and lectin from the seeds of *Abrus precatorius L.* and confirmed the blastogenic property of the abrus lectin cytologically and cytochemically. In the present study, the relationships among blastogenic agent, hemagglutinin and toxin were also discussed.

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Materials and Methods

Preparation of abrus lectin

Jequirity bean (*Abrus precatorius L.*) was obtained from Taiwan. Abrus lectin was prepared by the method as previously described (6). Our procedures partly differ from the method of Olsnes et al (7) in that the lectin was purified by passing it through a column of Sepharose 4B before using DEAE-cellulose chromatography to prevent the digestion of abrus lectin by proteases. Purification

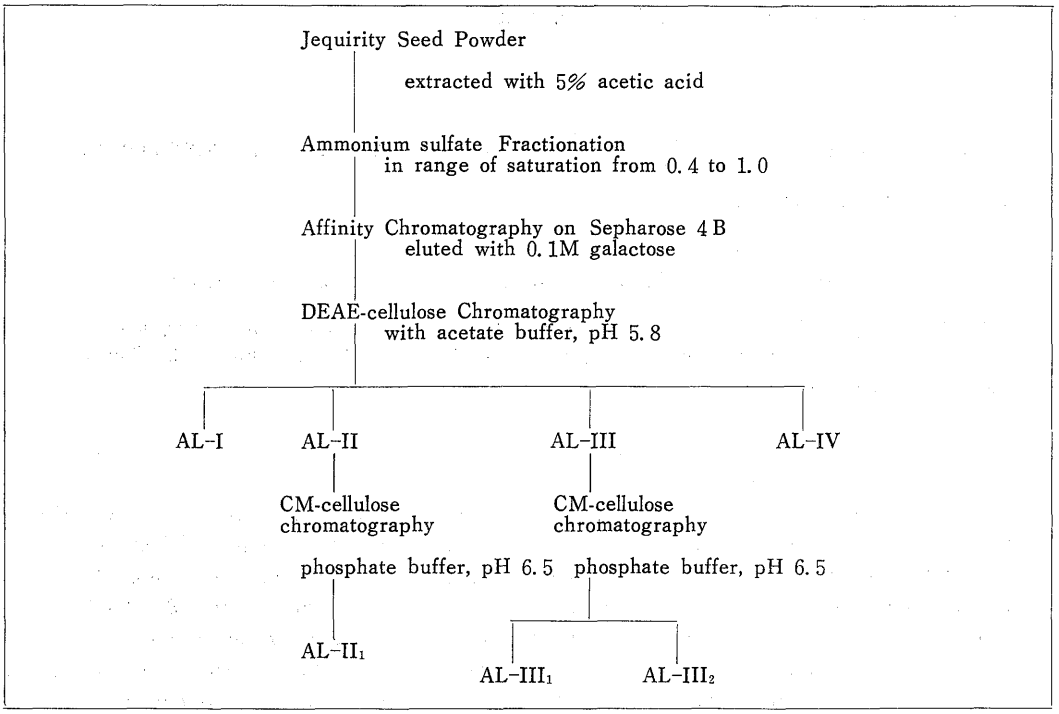

Fig. 1 Procedure for extraction and fractionation of abrus lectin

procedures of abrus lectin are presented in Fig. 1. In brief, the seeds of Jequirity bean were finely ground and the powder was suspended overnight in 5% acetic acid. The material was then centrifuged and the pellet was discarded. The supernatant was dialyzed with ammonium sulfate. The fraction, precipitated with ammonium sulfate at 0.4 to 1.0 saturation, was applied to a Sepharose 4B column equilibrated with 0.01M Tris-HCl buffer, pH 7.7, in 0.03M NaCl. The column was washed with the same buffer and the lectin was eluted with 0.1M galactose in the same buffer. The lectin was dialyzed against 0.005M acetate buffer, pH 5.8, and then absorbed on a DEAE-cellulose column. Four fractions (AL-I, II, III, IV) were eluted from the column with stepwise elution (0.005M to 0.2M acetate buffer) and, finally, with 0.2M acetate buffer, pH 5.8, plus 1.0M NaCl. Two fractions (AL-II, AL-III) were dialyzed with 0.01M phosphate buffer, pH 6.5, then absorbed on a CM-cellulose column. The lectins (three fractions, AL-II_1, AL-III_1 and AL-III_2) were eluted from the column with a gradient of NaCl (0 to 0.5M) in the same buffer. Quantitative estimation of extracted proteins in each purification procedure was performed at 280mμ and 260mμ with a spectrophotometer (Table 1). After dialysis against 0.9% NaCl, each fraction was filtered through Millipore filter (0.45μ) for use in the lymphocyte culture. The lectin (PHA-P, Difco) from *Phaseolus vurgalis L.* was reconstituted by adding 5ml of 0.9% NaCl.

Hemagglutinating test

Hemagglutinating activity was tested according to the method of Olsnes et al (7). Increasing amounts of lectin were mixed with 0.2ml of 4% human erythrocytes in 0.9% NaCl. The mixtures were incubated at 37°C for 60 min. The unit of hemagglutinating activity was defined as inverse number of the amount of lectin (mg/ml) needed to give a clearly visible agglutination of human erythrocytes.

Toxicity test

Some fractions (AL-II_1, AL-III_1, AL-III_2) were injected intraperitoneally into male mice of the

ddN strain and MLD_{48} was measured.

Disc gel electrophoresis

The isolated lectins were treated with sodium dodecylsulfate in the presence and absence of 1% β-mercaptoethanol and subsequently analyzed by polyacrylamide gel electrophoresis in 7.5% acrylamide gels.

Lymphocyte culture

Human peripheral blood lymphocytes were separated using the method of Böyum (8) modified by Kato (9). The lymphocytes (10^6/ml) were suspended in 1ml TC-199 with 20% calf serum and incubated with the abrus lectin or PHA in test tubes (14× 100mm) at 37°C in a 95% air-5% CO_2 humid atmosphere for 72 h. The percentage of blastoid cells was determined according to the morphological analysis as previously reported (9) and served as the index of the blastoid transformation. Cell viability of lymphocytes was tested according to the trypan blue dye exclusion method of McLimans et al (10). For this purpose 0.05ml of 0.5% trypan blue was mixed with 0.1 ml of cell suspension and placed in a hemocytometer, then, 500 cells, both dead and viable, were counted. The percentage of viable (nonstained) cell was taken as the index of cell viability.

Measure of isotope incorporation

The lymphocyte culture with the lectin was also performed in plastic test plates (Falcon Plastics) containing 2×10^5 cells in 0.2ml TC-199 with 20% calf serum at 37°C for 72 h. In order to investigate the effect of abrus lectin on DNA synthesis in lymphocytes, ^{3}H-TdR (1.0μCi/ml, 5Ci/mole, The Radiochemical Centre Amersham) was added to the culture medium (0.2ml) in each well 16 h prior to culture termination The cultured cells were harvested with a multiple cell harvester (Minimash AM 78, Dynatech Co.) and then radio activity was counted with a Packerd liquid scintillation counter.

Quantitative estimation of DNA

DNA in the lymphoid cells was demonstrated by the Feulgen reaction according to the method of Tomonaga et al (11). The content of Feulgen-DNA in the lymphoid cells, cultured with abrus lectin or PHA for 72 h, was measured at 560mμ with a scanning microspectrophotometer (Nikon-Vickers M85) by the method as previously reported (12). In each smear, 100 cells were examined and the mean value of Feulgen-DNA content was calculated.

Results

Purification of abrus lectin

Crude abrus lectin was purified first by chromatography on a Sepharose 4B because lectin has a high affinity to Sepharose 4B. The abrus lectin eluted with 0.1M galactose from Sepharose 4B column did not display protease activity. Our procedure for purification of abrus lectin was useful to prevent the digestion of the lectin by proteases contained in the protein. The abrus lectin which bind to a Sepharose 4B column was eluted by 0.1M galactose (Fig. 2, peak AL). The material which was not absorbed on Sepharose 4B had weak toxicity and hemagglutination. In the further purification the lectin in AL-fraction was applied to a DEAE-cellulose column. The absorbed proteins on a DEAE-cellulose column were elut-

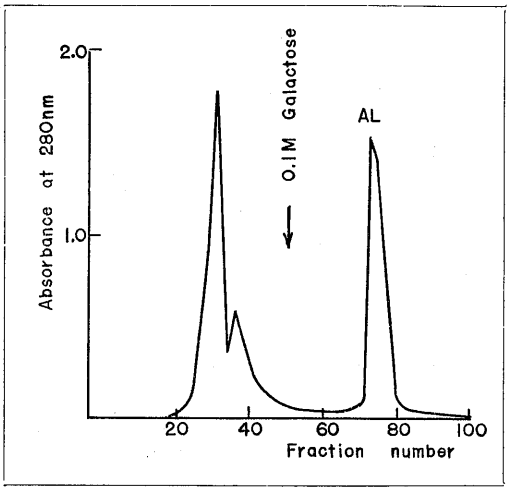

Fig. 2 Affinity chromatography of abrus lectin on a Sepharose 4B column. Fraction, precipitated with ammonium sulfate at 0.4 to 1.0 saturation, was applied to a Sepharose 4B column (3×23cm) equilibrated with 0.01M Tris-HCl buffer, pH 7.7, —0.03M NaCl. The column was washed with the same buffer and the lectin was eluted with 0.1M galactose in the same buffer.

ed with acetate buffer. Four fractions (AL-I, II, III, IV) were obtained with stepwise elution (Fig. 3). Two fractions (AL-II, AL-III) which possessed a very high hemagglutinating activity was chromatographed on a CM-cellulose column. Three fractions, AL-II_1 from AL-II and AL-III_1 and AL-III_2 from AL-III (Fig. 4) were eluted from the column with a gradient of NaCl.

Biological and chemical properties of abrus lectin

Hemagglutinating activity and toxicity of different fractions of abrus lectin were shown in Table 1. It is apparent that a AL-III_2 fraction which possessed a very high hemagglutinating activity (1000 unit/mg) had comparatively lower toxicity (MLD_{48}; 0.005 mg) than that of a AL-II_1 fraction in mice. The purified lectin (AL-III_2 fraction) was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

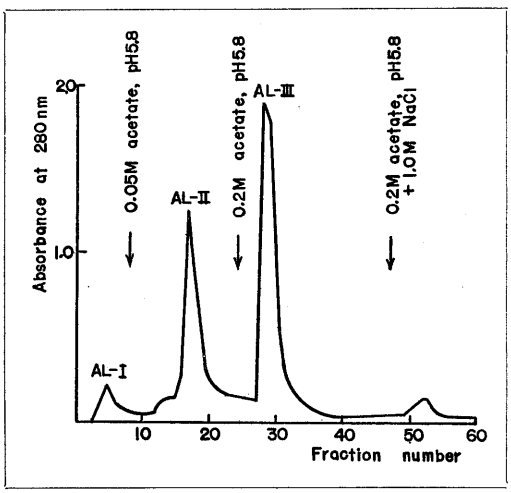

Fig. 3 DEAE-cellulose chromatography of abrus lectin (AL-fraction). AL-fraction (indicated in Fig 2) was dialyzed against 0.005M acetate buffer, pH 5.8 and then absorbed on a DEAE-cellulose column (1×10cm). The lectin was eluted from the column with stepwise elution. (0.005M to 0.2M acetate buffer) and finally with 0.2M acetate buffer, pH 5.8, +1.0M NaCl.

The abrus lectin had a molecular weight of 77,000. On treatment of β-mercaptoethanol, the lectin was split into two components with molecular weights of 40,000 and 36,000

Blastogenic properties of abrus lectin

In the abrus lectin-stimulated culture, both the AL-II_1 and AL-III_2 fractions induced the blastoid transformation of human blood lymphocytes, although its extent was considerably lower than that in the PHA culture (Table 2). The blastoid cells had a round nucleus with dispersed chromatin and one or more prominent nucleoli. Their cytoplasm was intensely basophilic and pyroninophilic. They resembled the blastoid cells in the PHA-stimulated culture in both size and staining characteristic (Fig. 5).

Incorporation of ^{3}H-TdR into the abrus lectin-cultured lymphocytes was 2,500 cpm in the AL-II_1 and 3,500 cpm in the AL-III_2 fraction. These were markedly low compared with the value of 11,255 cpm in the PHA-cultured lymphocytes. This closely paralleled the morphological evidence of transformation.

Relative amount of Feulgen-DNA in human lymphocytes cultured with abrus lectin at 37°C for 72 h was not as high as that of PHA culture, but distinctly 15% higher than that of the control (Fig. 6). These results indicate that abrus lectin also stimulates DNA synthesis in human lymphocytes in vitro.

The dose dependency of lymphocyte response to the abrus lectin was shown in Table 2. The blastoid transformation did not occur at concentrations of more than 10μg abrus lectin/ml because they were sufficiently cytotoxic to kill the lymphocyte in vitro. However, abrus lectin at lower concentrations (less than 1.0μg/ml) was clearly effective for blastoid transformation. The percentages of viable cells were 35.9% in the AL-II_1 culture and 56.5% in the AL-III_2 culture at the concentration 1.0μg/ml per culture. The maximal degree of blastoid

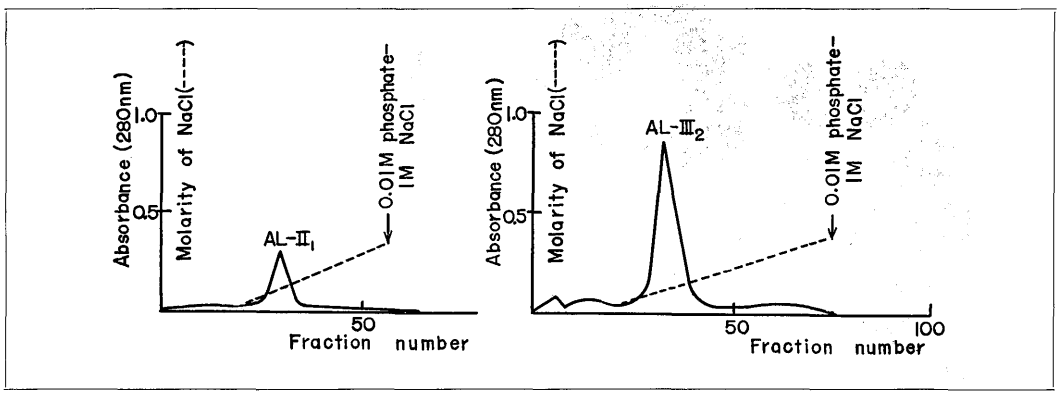

Fig. 4 CM-cellulose chromatography of abrus lectin. AL-II fraction (fraction No. 17 to 19) and AL-III fraction (fraction No. 28 to 31) was dialyzed against 0.01M phosphate buffer, pH 6.5 and then absorbed on a CM-cellulose column (1×10cm). The lectin was eluted from the column with a gradient of NaCl (0 to 0.5M) in the same buffer.

Table 1 Hemagglutinating activity and toxicity of different fractions

Fraction (mg)		Protein	Hemagglutinating activity (units/mg)	Toxity MLD_{48} (mg)
40-100% $(NH_4)_2SO_4$		234	140	—
Sepharose 4B (Fig. 2)		40	330	—
DEAE-cellulose (Fig. 3)	AL-I	—	—	—
	AL-II	6.2	920	—
	AL-III	15.0	670	—
	AL-IV	—	—	—
CM-cellulose (Fig. 4)	$AL-II_1$	3.4	500	0.003
	$AL-III_1$	0.5	200	0.025
	$AL-III_2$	12.0	1000	0.005

Hemagglutinating activity of 1mg PHA-P (Difco) is 400 units.
MLD_{48}: Minimum lethal dose after 48 h

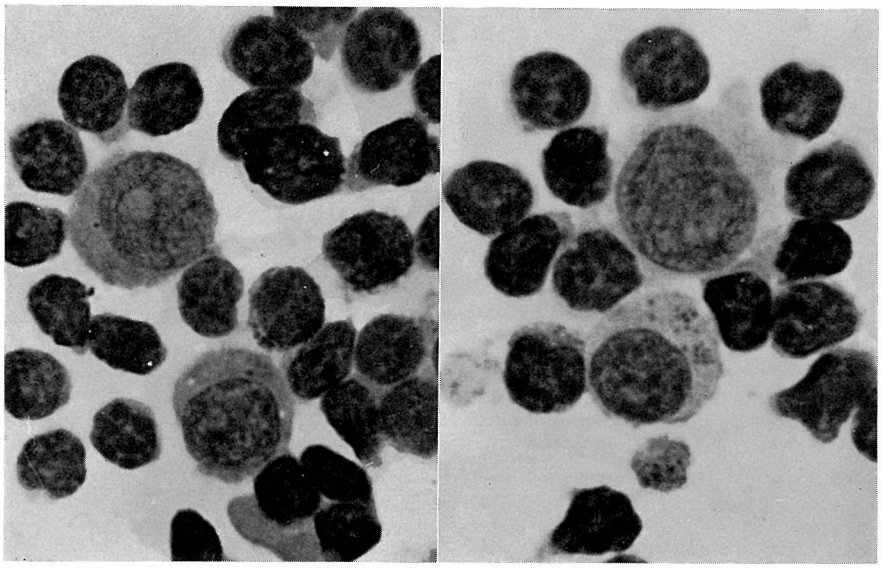

Fig. 5 Lymphoid cells in smears from blood lymphocytes, obtained from each of two donor, cultured for 72 h with abrus lectin (AL-III$_2$ fraction 1.0 μg/ml). Note typical blastoid cells. Wright's stain ×1000

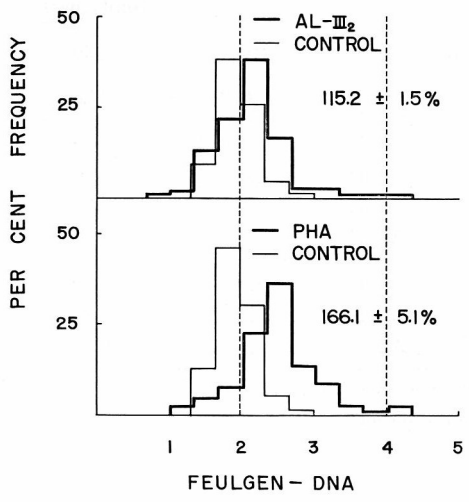

Fig. 6 Histogram of relative amount of Feulgen-DNA in lymphoid cells cultured with abrus lectin (AL-III$_2$ fraction) or PHA at 37°C for 72 h. Numbers show percentages of control. (Mean ± standard error, n=100).

Table 2 Percentages of blastoid cells in human blood lymphocytes cultured with abrus lectin or PHA at 37°C for 72 h

Concentrations of lectins (μg/ml)	Blastoid cells (%)		
	AL-II_1	AL-III_2	PHA
100	0	0	44.8
10	0	0.3	61.6
1.0	4.5	6.4	43.5
0.1	2.1	5.7	6.6
0.01	0.9	3.9	0.4
0.001	0.8	0.3	N.D.

AL : Abrus lectin
PHA : Phytohemagglutinin (PHA-P, Difco)
N.D. : not done

transformation was observed to be 6.4% in the presence of 1.0μg abrus lectin (AL-III_2)/ml, which has high hemagglutinating activity. On the other hand, PHA constantly produced higher percentage (43.5%-61.6%) of blastoid cells in concentrations from 1.0 to 10μg/ml.

Discussion

The blastoid transformation of human blood lymphocytes was observed in abrus lectin-stimulated cultures although blastogenic activity of this lectin was lower than that of PHA. This lower activity is similar to that of *Phaseolus sp.* (Japanese red bean) as shown by Parker et al (13). The abrus lectin-induced blastoid cells resembled PHA-induced blastoid cells in both size and staining characteristics. In accordance with the morphological evidence of transformation, a slightly enhanced incorporation of ^{3}H-TdR and an increase of Feulgen-DNA content in lymphocytes were demonstrated in abrus lectin-stimulated cultures. Closs et al (5) reported that abrus lectin stimulated a lymphocyte population where less than 2% of the cells contained surface immunoglobulin. These results indicate that abrus lectin may be T lymphocyte mitogen.

Hemagglutinating activity of abrus lectin increased with its progressive purification. Concerning the effect of hemagglutination on blastogenesis, Parker et al (13) reported that the degree of blastogenesis in lymphocyte culture is enhanced by increased cell density or agglutination. This may be related to the finding that the AL-III_2 fraction, which possesses a higher hemagglutinating activity, induces a relatively high activity in blastogenesis of human blood lymphocytes. Downing et al (14) suggested that the mitogenic factor in some plant lectins may be independent of hemagglutinating activity. The lectin which was low hemagglutinating activity and high mitogenic properties had been purified from *Phaseolus vulgaris* by Allen et al (15). Thus, mitogenic agent may fundamentally differ from hemagglutinating agent.

We observed that the lectin (AL-III_2 fraction) which possessed higher hemagglutinating activity had lower toxicity as compared with the lectin (AL-II_1 fraction). More recent workers have indicated that the toxin and the hemagglutinating properties of plant lectins, *Abrus precatorius* and *Ricinus communis* (7, 16), belong to separate proteins. However, the relationships among mitogenecity, hemagglutination and toxicity are still obscure. The cytotoxicity of lectin preparations may be due to contamination by traces of residual toxins. Thus, highly purified abrus lectin in further experiments may show a much low toxicity and higher blastogenic activity than in the present experiment. Further purifications of abrus lectin involving this point are now in progress in our laboratory.

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