Purification and Characterization of Abrus precatorius Agglutinin

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Purification and Characterization of *Abrus precatorius* Agglutinin

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Abrus precatorius agglutinin (APA) has been purified by a new purification procedure from the seeds of semen jequiriti produced in Bangladesh and Taiwan. The method was accomplished by 33-50% saturation ammonium sulfate fraction from 1% acetic acid extract of the seeds of semen jequiriti using gel filtration on Sephadex G-75 followed by DEAE-cellulose column chromatography. The molecular weight was estimated to be 126,000 and 122,000 by gel filtration on Sephadex G-150 for Bangladesh-APA and Taiwan-APA, respectively. Both the APA were found to be consist of *two* types of polypeptide chains of nearly same size (30,000 to 34,000), which possess valine and proline as N-terminal amino acid. Furthermore, one of the constituent polypeptide chains of APA showed microheterogeneity, suggesting that APA is a mixture of isolectins.

INTRODUCTION

Abrus precatorius agglutinin has been prepared and characterized by several laboratories. It has been reported that APA is a tetrameric lectin with molecular weight of 130,000 and consists of two pairs of non-identical polypeptide chains, A- and B-, bridged by disulfide bond (Olsnes, 1978; Kaufman and McPherson, 1975). Olsnes *et al.* (1974) indicated that APA is heterogeneous and consists of two populations of molecules which differ only in their heavy peptide chains (probably B-chains) whereas Wei *et al.* (1975) reported that APA is homogeneous and consists of four polypeptide chains (2 A-chains and 2 B-chains), of which two pairs have either identical or closely similar molecular sizes (33,800 and 32,000). Recently, Lin *et al.* (1981) reported APA as a dimer with molecular weight of 67,000.

To obtain more information on the nature of APA, in this communication, we have purified APA from the seeds of semen jequiriti produced in Bangladesh and Taiwan, Republic of China, using gel filtration on Sephadex G-75 column followed by DEAE-cellulose chromatography and investigated some of its properties.

MATERIALS AND METHODS

Seeds of semen jequiriti were collected from Bangladesh and Taiwan, Republic of China. Sephadex G-150, Sephadex G-75, DEAR-cellulose and Sepharo-

se 4B were purchased from Pharmacia Fine Chemicals, Upsala, Sweden. Galactosamine-Cellulofine was obtained from Chisso Co., Tokyo.

Preparation of crude extract

Decorticated *Abrus* seeds were suspended in 5% acetic acid and allowed to soak overnight at 4°C. The soaked seeds were cut into small pieces and finely homogenized with a homogenizer in 1 % acetic acid. The homogenate was centrifuged at 10,000 *g* for 20 minutes and the supernatant was made 100 % saturation with ammonium sulfate. The resulted precipitate was collected by centrifugation and dialyzed against deionized water for 24 hours. After centrifugation, saturated ammonium sulfate solution was added to the supernatant and the precipitate obtained from 33-50 % ammonium sulfate saturation was used as crude lectin.

Affinity chromatography

Affinity chromatography was carried out on Sepharose 4B or galactosamine-Cellulofine column as previously described (Funatsu et al., 1977). After dialysis against 5 mM phosphate buffer saline, pH 7.1, the lectin was applied to Sepharose 4B or galactosamine-Cellulofine column previously equilibrated with the same buffer and developed with the same buffer at 4°C. The adsorbed lectin was eluted with the same buffer containing 0.1 M lactose.

Electrophoresis

Polyacrylamide disc gel electrophoresis was conducted at room temperature, pH 8.5, on 7.5 % gels as described by Ornstein (1964) and the protein was stained with amido black.

Cytoagglutinating activity

Cytoagglutinating activity was determined by using Sarcoma 180 ascites tumor (SAT) cells or human O-type red blood cells as previously described (Funatsu et al., 1976; Lin et al., 1981). 0.2 ml of lectin solution with various concentrations, which had been previously dialyzed against 5 mM phosphate buffer saline, pH 7.1, was added to 0.2 ml of SAT cells (1 x 10' cells/ml) suspension or 2 % washed red blood cells suspension and incubated for 20 minutes or 2 hours, respectively. The agglutinating potency was measured under a microscope in SAT cells and visually by observing the pattern formed by the agglutinated cells on the bottom of the tube in red blood cells and expressed as minimum amount necessary to agglutinate a major part of the cells in small aggregates.

Ultraviolet absorption spectroscopy

The ultraviolet absorption spectrum of lectin was recorded in an aqueous solution with a Hitachi Model 200-10 Spectrophotometer.

Molecular weight estimation

The molecular weight was determined by gel filtration on Sephadex G-150 according to Mazumder et al. (1981) and by SDS-polyacrylamide gel electro-

phoresis as described by Weber and Osborn (1969). A column of Sephadex G-150 was equilibrated with 10 mM Tris-HCl buffer containing 0.1 M NaCl, pH 7.8 at 4°C using castor bean hemagglutinin (CBH), ricin D and lysozyme as reference proteins. The elution volume was measured from the position of the maximum height of the elution profile.

Determination of molecular weight was performed by SDS-polyacrylamide electrophoresis using 10 % gel. The marker proteins used are CBH-A chain, CBH-B chain, catalase, α-chymotrypsinogen, ovalbumin and lysozyme.

Amino acid analysis

The purified lectin was hydrolyzed with 6 N HCl containing 0.05 % 2-mercaptoethanol in sealed and evacuted tubes for 24 hours at 110°C and was analyzed with a JEOL amino acid analyzer (Type GLC-6AH). Tryptophan was determined by N-bromosuccinimide oxidation according to Spande and Witkop (1967).

N-terminal amino acid

N-terminal amino acid of APA was analyzed by DABITC/PITC double coupling method as previously described (Chang et al., 1978).

RESULTS

Purification of APA

The crude lectin from the seeds produced in Bangladesh or Taiwan was applied to Sephadex G-75 column at 4°C previously equilibrated with 50 mM borate buffer, pH 8.0, and eluted with the same buffer. As shown in Fig. 1, both crude lectins were eluted as two peaks, fraction F1 and F2, at the positions corresponding to molecular weights of near 120,000 and 60,000 ; respectively. Since it is evident that fraction F1 and F2 contain aggulutinin and abrin, respectively from their molecular weights, the fraction F1 was collected and further purified by DEAE-cellulose chromatography. The fraction F1 was precipitated with solid ammonium sulfate and the precipitate was dissolved in deionized water, dialyzed against deionized water and 5 mM Tris-HCl buffer, pH 8.5 at 4°C. After removal of the insoluble material, the clear supernatant was applied to DEAE-cellulose column at 4°C, previously equilibrated with 5 mM Tris-HCl buffer, pH 8.5 and eluted by a linear gradient of NaCl from 0 to 0.2 M in the same buffer. As observed in Fig. 2, the protein was eluted as a single but broad peak, indicating the presence of more than one component. In order to separate these components, the elution was carried out stepwisely with increasing concentrations of NaCl in the same buffer. As shown in Fig. 3, the fraction F1 from Taiwan seeds were separated into four fractions, fraction-a, -b, -c and -d, whereas that of Bangladesh seeds into two fractions, fraction-a and -b. On polyacrylamide disc electrophoresis, as shown in Fig. 4, all the fractions gave single band with almost identical mobilities at pH 8.5.

Cytoagglutinating activities of these fractions were determined using SAT

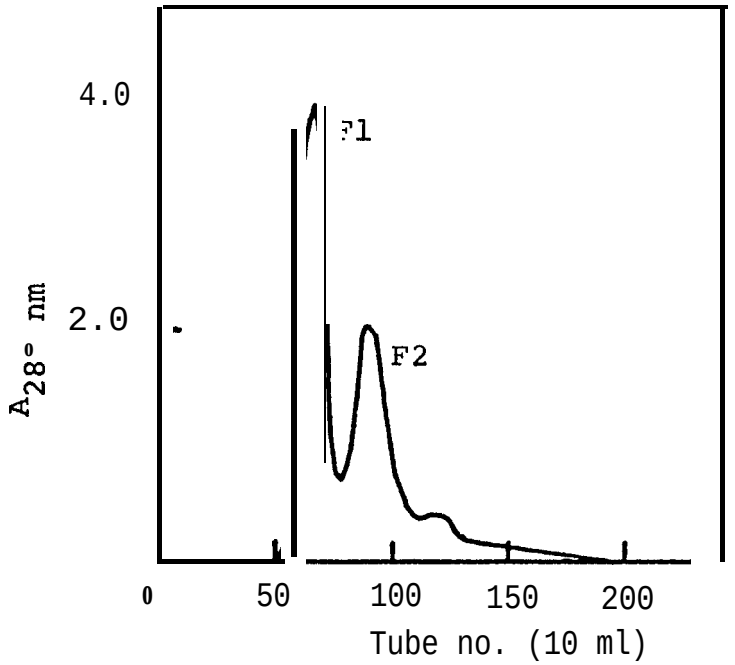


Fig. 1. Gel filtration of crude extract of Bangladesh-APA or Taiwan-APA through Sephadex G-75. The crude extract was applied to the column (5.4~ 132 cm) of Sephadex G-75 preequilibrated with 50 mM borate buffer, pH 8.0, 4°C and developed with the same buffer.

cells and it was found that fraction-a and -b from Bangladesh seeds and fraction-b and -c from Taiwan seeds possessed the highest cytoagglutinating activities (Table I). Their ultraviolet absorption spectra also showed the same typical protein pattern having maximum and minimum at 280 nm and 250 nm, respectively (Fig. 5), however, the absorption spectra of fraction-a and -d from Taiwan seeds obtained are significantly different from those of the other fractions with maximum and small minimum peak at 272 nm and 252-54 nm, respectively (Figures, not shown).

The fraction-a from Bangladesh seeds and fraction-b from Taiwan seeds, eluted by the same salt concentration in highest yield were used as *Abrus precatorius* agglutinin (APA) and referred to as Bangladesh-APA and Taiwan-APA, respectively, and used for all further studies.

Properties of APA

i) Molecular weight: The molecular weight of Taiwan-APA and Bangladesh-APA were determined by gel filtration through Sephadex G-150 at pH 7.8 and calculated to be 122,000 for the Taiwan-APA and 126,000 for the Bangladesh-APA (Fig. 6a and 6b).

ii) Hemagglutinating activity : The Bangladesh-APA and Taiwan-APA are able to cause the agglutination of human red blood cells (O-type) at 0.05 μg/

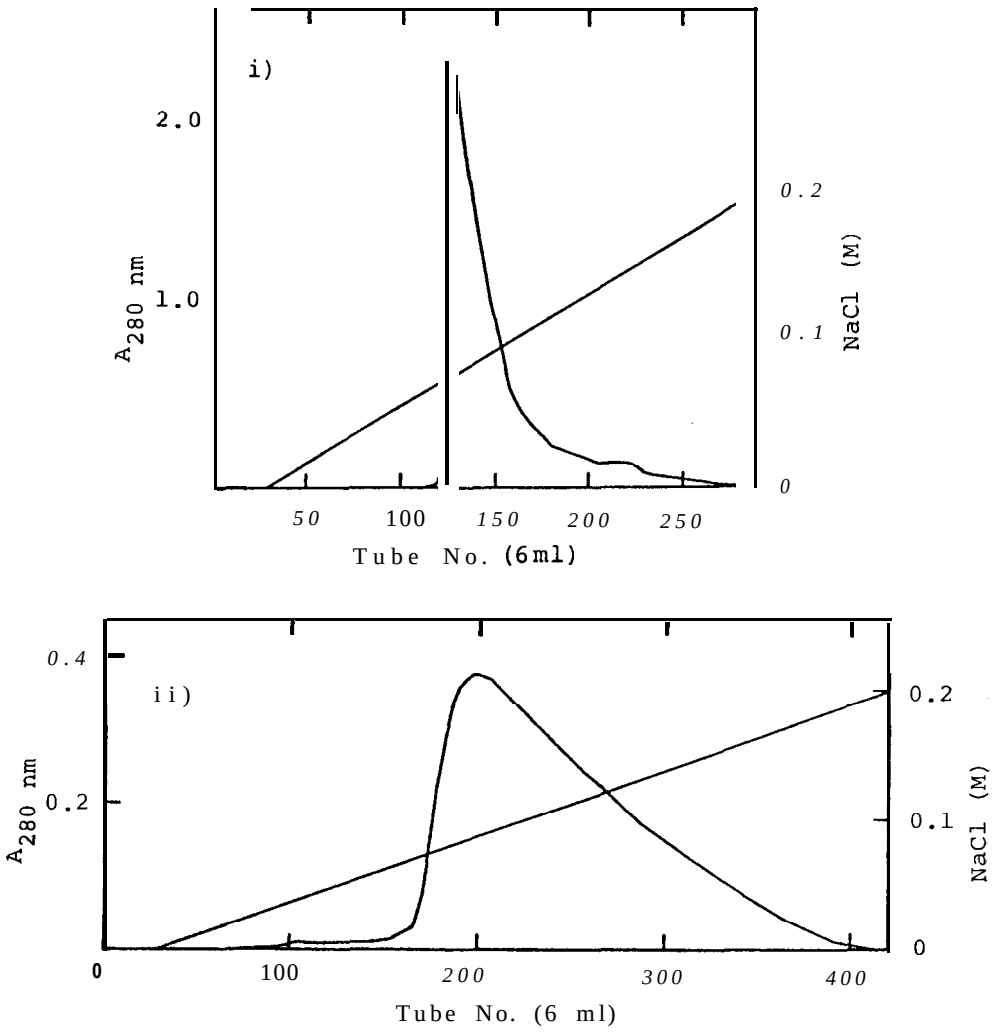


Fig. 2. DEAE-cellulose chromatography of F1 fraction. F1 fraction obtained from, *i)* Bangladesh seeds (250 mg) and ii) Taiwan seeds (190 mg), by gel filtration, were applied to DEAE-cellulose column (2.1 X34 cm) prewashed with 5 mM Tris-HCl buffer, pH 8.5 at 4°C and eluted by a linear gradient of NaCl (0 to 0.2M) in the same buffer.

ml concentration.

iii) Binding ability: The binding ability of Taiwan-APA and Bangladesh-APA to Sepharose 4B and galactosamine-Cellulofine were examined by chromatography. Both the agglutinins bound firmly to the Sepharose 4B or galactosamine-Cellulofine in phosphate buffer saline, pH 7.1 at 4°C and eluted as a single sharp peak by 0.1 M lactose in the same buffer (Fig. 7), indicating that

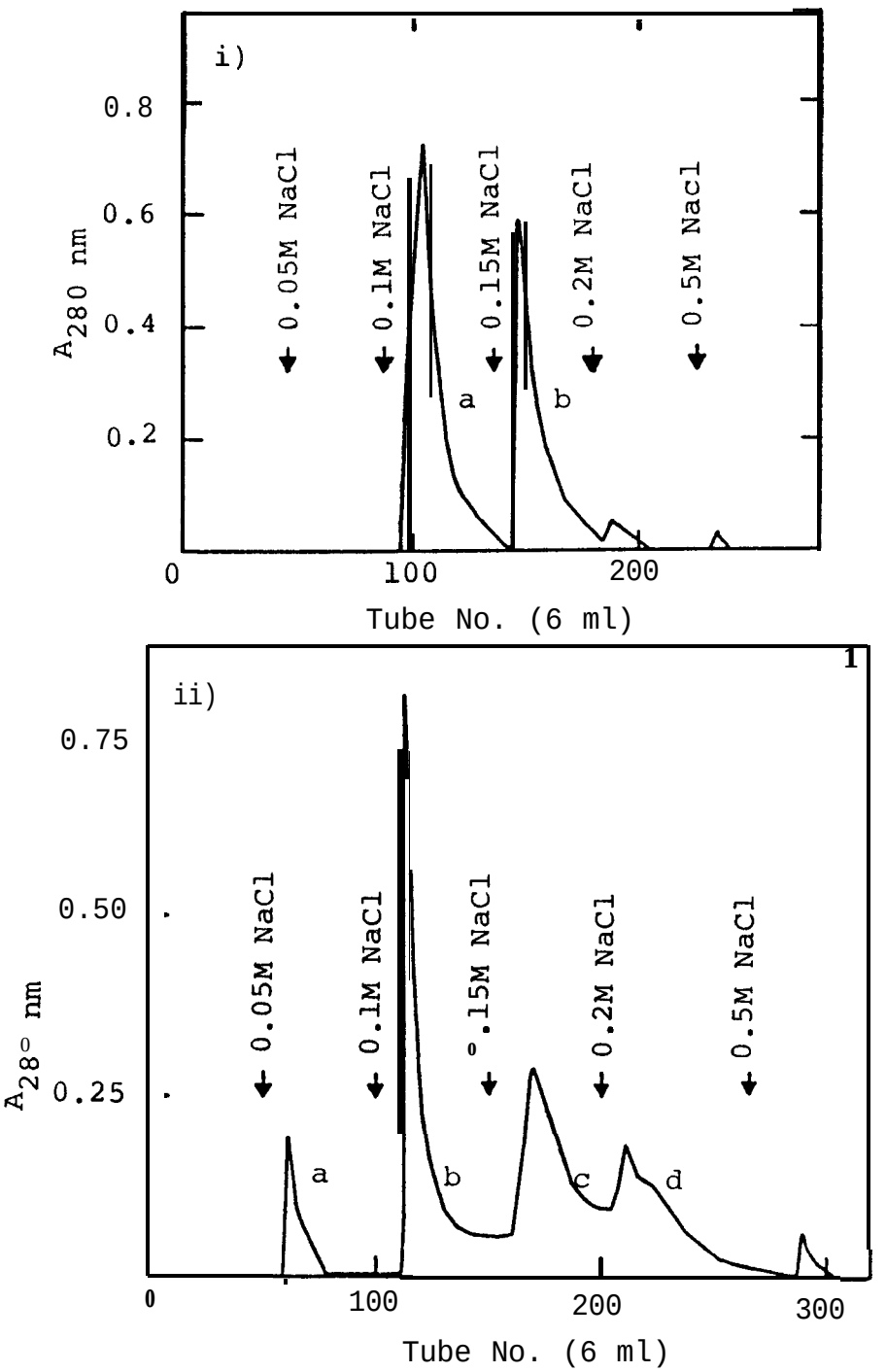


Fig. 3. DEAE-cellulose chromatography of F1 fraction. F1 fraction obtained from, i) Bangladesh seeds (195mg) and ii) Taiwan seeds (130 mg) by gel filtration, were applied to DEAE-cellulose column (2.1 x 34 cm) prewashed with 5mM Tris-HCl buffer, pH 8.5 at 4°C and eluted by stepwise increases of NaCl concentrations in the same buffer.

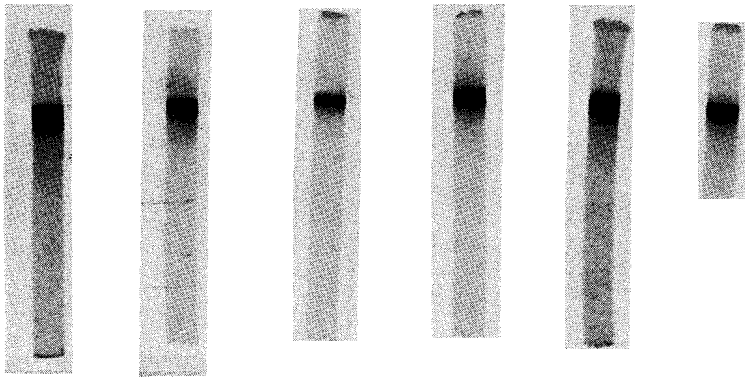


Fig. 4. Polyacrylamide disc gel electrophoresis of the different fractions of Bangladesh-APA and Taiwan-APA obtained from DEAE-cellulose chromatography by stepwise elution of increases of NaCl concentrations. Gel concentration was 7.5%. Bangladesh-APA: i) fraction-a and ii) fraction-b, Taiwan-APA: iii) fraction-a, iv) fraction-b, v) fraction-c and vi) fraction-d.

Table I. Agglutinating activity of APA and different fractions of APA

Fraction	Agglutinating activity		
	SAT cells		O-type red blood cells
	Minimum conc. $A_{280\ nm}$	(%)	Minimum conc. (μg/ml)*
Bangladesh-APA	0.0030	(100)	0.05
Taiwan-APA	0.0030	(100)	0.05
DEAE-cellulose:			
Bangladesh-APA, a,	0.0030	(100)	
〃 , b,	0.0030	(100)	
Taiwan-APA , a,	0.010	(30)	
〃 , b,	0.0030	(100)	
〃 , c,	0.0030	(100)	
〃 , d,	0.0060	(50)	

* Concentration of protein was determined according to Olsnes *et al. (1974).*

the agglutinin binds to galactose as well as galactosamine.

Amino acid composition and N-terminal amino acid of APA

The total number of amino acid residues per APA molecule were calculated by taking the value of histidine, which is present in least amount, 12.1, as reported by Wei *et al.* (1975). The amino acid composition of Bangladesh-

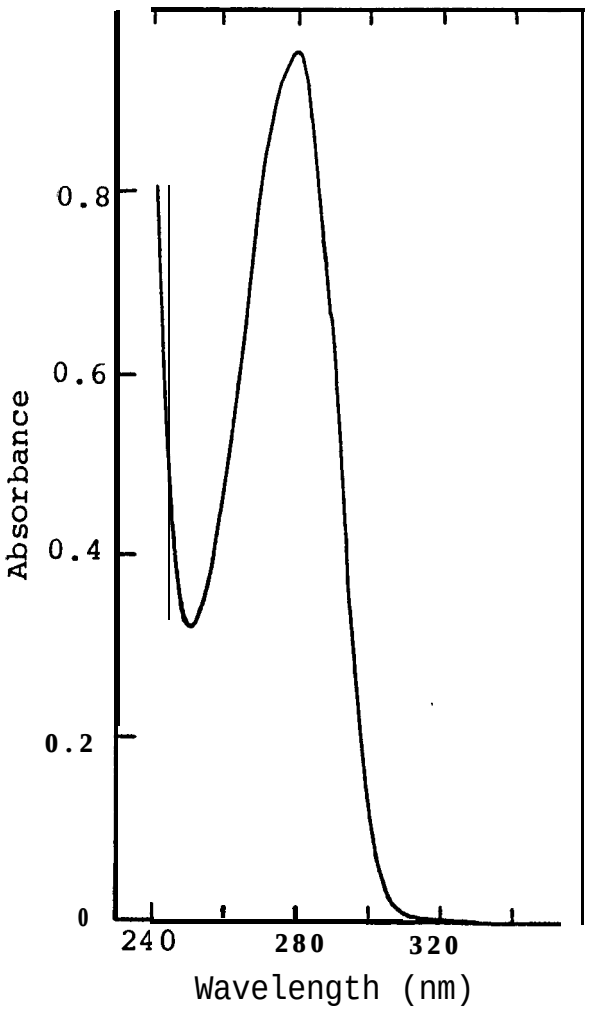


Fig. 5. Ultraviolet absorption spectrum of Bangladesh-APA or Taiwan-APA. The ultraviolet spectrum was determined in an aqueous solution.

APA and Taiwan-APA are given in Table II together with that of the *Abrus* agglutinin reported by Wei et *al.* (1975). The amino acid composition of Bangladesh-APA and Taiwan-APA are very similar, but distinctly differ in Asp, Pro and Val.

The N-terminal amino acids of Bangladesh-APA and Taiwan-APA were identified to be valine and proline.

Subunit structure of APA

Subunit structure of APA was examined by SDS-polyacrylamide gel electrophoresis. When APA was incubated with 1% SDS at room temperature for overnight, Bangladesh-APA and Taiwan-APA gave a single band with mo-

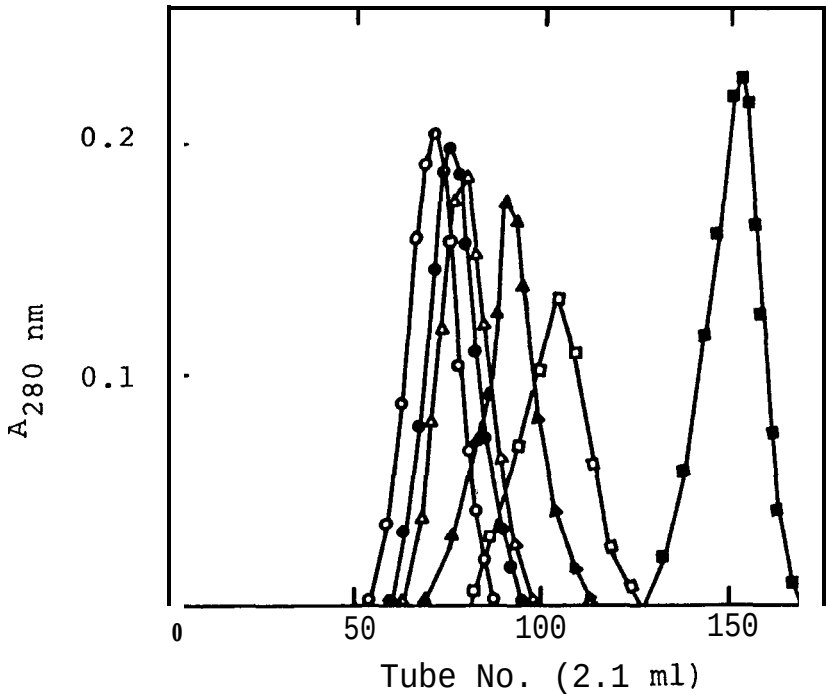


Fig. 6a. Gel filtration of APA and different reference proteins through Sephadex G-150. Proteins were applied to a column of Sephadex G-150 (1.9 ×87 cm) at 4°C, pre-equilibrated with 10 mM Tris-HCl buffer, pH 7.8 containing 0.1 M NaCl or 0.1 M acetate buffer, pH 4.0, and developed with the same buffer. (●) Bangladesh-APA, pH 7.8; (○) CBH, pH 7.8 or pH 4.0; (□) Ricin D, pH 7.8 or pH 4.0; (△) Taiwan-APA, pH 7.8; (A) Bangladesh-APA or Taiwan-APA, pH 4.0 and (■) Lysozyme, pH 7.8.

lecular weight of about 64,500 and 63,000, respectively. On the other hand, when treated with 1% 2-mercaptoethanol in the presence of 1 % SDS, the Bangladesh-APA was converted into three bands while that of Taiwan-APA gave two bands, of which, the band of its heavy chain (B-chain) was more broader than that of Bangladesh-APA (Fig. 8). Their molecular weights were estimated to be 34,000, 33,000 and 31,000 for Bangladesh-APA and 33,000 and 30,000 for Taiwan-APA (Fig. 9).

DISCUSSION

The data presented in this communication clearly demonstrated that *Abrus* agglutinin is heterogeneous and consists of two populations of molecules which differ only in their heavy chains (B-chain). It was also found that agglutinin in the seeds of semen jequiriti produced in Bangladesh and Taiwan are heterogeneous and can be seperated by DEAE-cellulose chromatography with stepwise increases of NaCl concentrations.

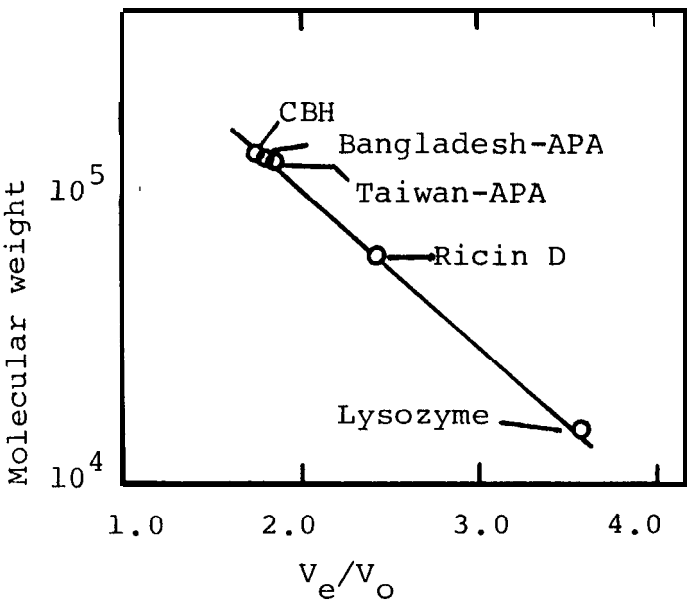


Fig. **6b.** Standard curve for molecular weight determination by gel filtration on Sephadex G-150. Chromatographic conditions are same as in Fig. **6a.** The elution volume (V_0) was measured from the position of the maximum height of the elution profile.

The polyacrylamide gel electrophoresis pattern of APA obtained in the presence of 1 % SDS clearly revealed that the intact agglutinin consisted of two complexes with molecular weight of 63,000–64, 500. This result suggests that the two A-B units of APA might be held together by weak noncovalent interactions. This result is similar to the findings of Olsnes *et al.* (1974). As shown in Fig. 8, the polyacrylamide gel electrophoresis pattern obtained with 2-mercaptoethanol in the presence of 1 % SDS for APA, revealed that Bangladesh-APA are converted into three bands (intensity of the slow moving band is very light than the other two) whereas Taiwan-APA are converted into one broader band (probably slow moving band i. e. broader band is composed of two thick bands of equal intensity) and one sharp band. This observation suggests that one of the polypeptide chains of APA is of heterogeneous nature.

As found, APA was transformed to dimer in the presence of SDS, so the molecular weight of intact APA was determined by gel filtration at pH 7.8, and found to be 126,000 and 122,000 for Bangladesh-APA and Taiwan-APA, respectively. This value is slightly smaller than that reported for APA by other authors (Wei *et al., 1975* and Olsnes *et al.,* 1974). Lin *et al. (1981)* identified APA with a molecular weight of 67,000 by SDS electrophoresis. As shown in Fig. 6a, the molecular weight of APA at pH 4.0 was found to be about 95,000 by gel filtration. This observation is not clearly understood at this moment, but we think that APA may undergo dissociation in the acidic pH.

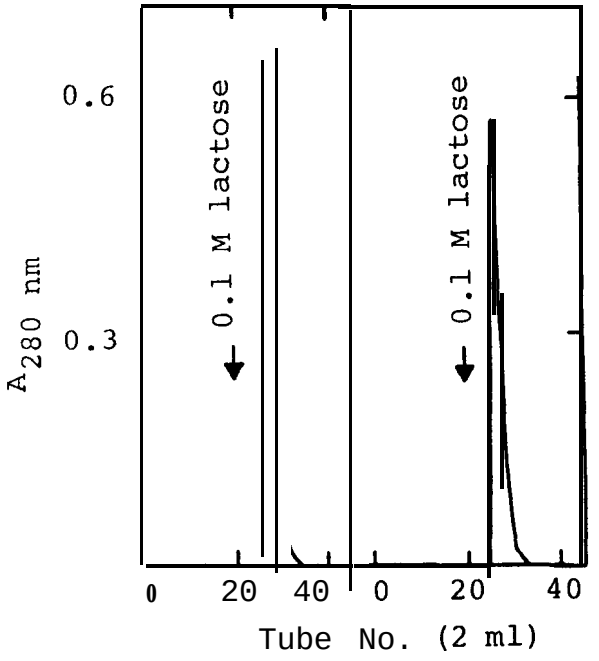
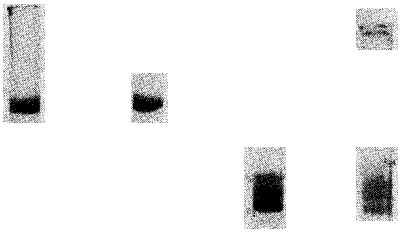


Fig. 7. Affinity chromatography of Bangladesh or Taiwan-APA (4mg) on a Sepharose 4B or galactosamine-Cellulofine column. The column was equilibrated with 5mM phosphate buffer saline, pH 7.1 at 4°C and the adsorbed protein was eluted with 0.1 M lactose in the same buffer. i) Sepharose 4B and ii) Galactosamine-Cellulofine. Column : (1.2~ 18 cm).



(a) (b) (c) (d)

Fig. 8. SDS-polyacrylamide gel electrophoresis of Taiwan-APA and Bangladesh-APA. Gel concentration was 10%. (a) Bangladesh-APA, (b) Taiwan-APA, (c) Bangladesh-APA in the presence of 1% 2-mercaptoethanol and (d) Taiwan-APA in the presence of 1% 2-mercaptoethanol.

Table II. Amino acid composition of *Abrus* agglutinin

Amino acid	Bangladesh-APA		Taiwan-APA		*APA
	No. of residues per histidine	No. of residues per APA	No. of residues per histidine	No. of residues per APA	
Lysine	3.21	38.84	3.20	38.82	38. 1
Histidine	1.00	12. 1	1.00	12.1	12. 1
Arginine	4. 13	49.97	4.11	49.73	50.6
Aspartic acid	10.18	123.18	10.85	131.28	127.4
Threonine	6.13	74.17	6.16	74.53	73. 5
Serine	7. 73	93.53	7.64	92.44	104.2
Glutamic acid	8. 57	103.70	8.48	103.61	107.7
Proline	3.85	46.59	4.42	53.48	50.9
Glycine	5. 56	67.27	5.66	68.48	67. 9
Alanine	6. 47	78.29	6. 47	78.29	76. 4
Valine	6.24	75.50	5.84	70.66	81.6
Methionine	1.72	20.81	1.55	18.76	*18. 3*
Isoleucine	4. 90	59.29	4. 67	56.51	56. 5
Leucine	7.04	85.18	6. 96	84.22	89.7
Tyrosine	3. 66	44.28	3.68	44.52	45. 0
Phenylalanine	2. 70	32.67	2.83	34.24	37.7
Cysteic acid	N. D.	N. D.	N. D.	N. D.	21. 6
Tryptophan[a]	1.98	23.67	N. D.	N. D.	20.3

N. D, Not detected
* As reported by Wei *et al. (1975)*
a According to Spande and Witkop (1967)

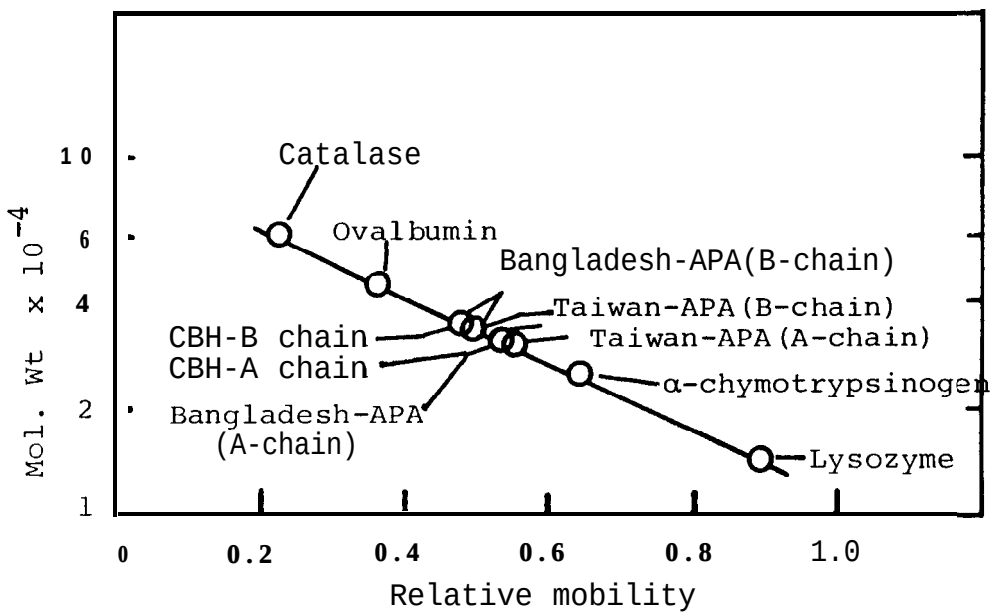


Fig. 9. Standard curve for molecular weight determination by SDS-polyacrylamide gel electrophoresis. Gel concentration was 10%. Mobilities were calculated relative to that of bromophenol blue.

Agglutinin was prepared from two different sources by the same procedure but the crude extract of two different sources showed different properties. As observed in Fig. 2 and 3, the elution profile of the crude extract of two different sources through DEAE-cellulose column by a linear gradient of NaCl or by stepwise elution with increasing concentrations of NaCl were differed significantly. From the elution profile of DEAE-cellulose chromatography, we suggest that agglutinin produced in *Abrus* seeds are of heterogenic nature.

Both the agglutinins were found to be bind to Sepharose 4B or galactosamine-Cellulofine even at room temperature, indicating that the agglutinin binds to galactose and galactosamine. However, it was found that the closely related *Ricinus* agglutinin bound only to Sepharose 4B but not to galactosamine-Cellulofine, indicating that the agglutinin binds to galactose but not to galactosammine. This is a remarkable difference between the two closely related agglutinins.

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