Iranian J. Publ. Hlth.
Atumn, 1979, Vol. 8, No. 3

ISOLATION AND PURIFICATION OF LECTIN FROM IRANIAN RICINUS COMMUNIS* SEEDS

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ABSTRACT

Iranian castor bean lectin was isolated and purified by several column chromotography methods. Then, the suger and amino acid content of the purified lectin was identified. Castor bean lectin has some anti tumor and anti leukemia action. Thus it may be used commercially for medical and biochemical purposes.

INTRODUCTION

Several lectins are shown to specifically aggulitinate certain viral transformed cell lines at concentrations which do not cause agglutination of the normal untransformed cell lines from which they were derived (1). Nicolson and Blaustein (2) reported the presence of two lectins with molecular weight of 60,000 (RCA60) and 120,000 (RCA120) in Ricinus communis seeds. They also reported that each of these lectins could be used to suppress growing mammary tumors in rats. RCA60 has been previously isolated from Iranian *R. communis* (3). In this paper we report on the presence, isolation, purification and some properties of RCA120 in these seeds.

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MATERIALS AND METHODS

Agglutination test: One drop of fresh human blood was added to a drop of seed extract on a slide, and agglutination was observed at room temperature. This test was used to detect activity of the seeds and the extracts.

Protein concentration: Protein concentration was measured by Biuret (4), using bovine serum standard curve and Beckman Model DB-GT spectrophotometer.

Specific activity: A convenient hemagglutination procedure was to prepare serial dilution of the lectin solution with 0.9% NaCl. A drop of 4% suspension of the fresh human blood was added to each dilution and hemagglutination plates were kept at room temperature for 15 min. Specific activity was the lowest concentration of lectin that agglutinated blood (5).

Suger analysis: Purified lectin was hydrolysed in the presence of sulfuric acid in vaccum for three h (6). Excess acid was removed by addition of barium hydroxide. The supernatant was used for two demensional thin layer chromatography on precoated plastic sheets (Polygram Sil G Machery Nagel and Co., Duren, Ger,). The first developing solution was isopreopanol and water (4:1 volume basis) and the second solvent was n-butanol, ethyl acetate, isopropanol, acetic acid and water (35: 100,: 60; 35:30 volume basis). To observe the spots, plates were sprayed by 0.2% naphtoresorcinol and 2% sulfuric acid in ethanol. The test spots were compared with standard suger prepared by the same method.

Amino acid analysis: Purified lectin was hydrolysed in the presence of 6 N HCl in vacuum for 24 h. Excess acid was evaporated and amino acids were analysed by JLA (Hitachi, Japan) automatic amino acid analyser based on the method of Spackman *et al* (7).

Disc electrophoresis: Electrophoresis was carried out according to the method of Davis and Ornstein (8) at PH 8.3 and 15 mA for 3 h. in Diac Electrophoresis Trennkammer DEA 90 (Lab. Sup. Co. Ollman and COKG, Ger.) Sephadex and agarose gels were obtained from Bio-Rad (Richmond, Calif.) Castor bean were a hand picked harvest of 1974 cultivated at Mahabad (west of Iran) for oil production.

Lectin extraction procedure: Lectin extraction was carried out by

ACKNOWLEDGEMENT

The authors wish to thank The Ministry of Science and Higher Education Funds for Financial supports.

Table I Specific activities during the course of purification

Extracts	Protein con. g/ml
A Agarose	0.5
B Sophadex G-100	0.4
C Sephadex G-150	0.1

modified method of Nicolson and Blaustein (2). Hulled ground seeds were extracted in ten parts of 5 mM sodium phosphate buffer, pH 7.2, for three h. The supernatant was then brought to 0.6 saturation with ammonium sulfate. To remove excess salt the clear supernatant which was active in agglutination test, was dialyzed against phosphate buffer for 21 h. The dialysate was concentrated by air blow at 4^{c}. The extract was then loaded on a column of A- 0.5 m· Agarose gel (4X 40 cm), previously equilibrated with $5m^{M}$ sodium phosphate buffer, pH 7.2. The column was washed with 2 to 3 liters of the same buffer and lectin was eluted by 0.25^{M} D-lactose in $5m^{M}$ phosphte buffer pH 7.2.

RESULT AND DISCUSSION

The elution profile after lectin extractions containing agglutinine activity were pooled and concentrated (A). The concentrated crude lectin was added to a sephadex G-100 column (2X 40 cm) previously equilibrated by $5m^{M}$ phosphate buffer, pH 7.2 Fig. 2 indicates the elution pattern of these fractions. Active fractions (B) were pooled again and for further purification were loaded on a sephadex G- 150 column (2x 40 cm). Fig. 3 shows this chromatogram. Specific activities during the course of purification were calculated and are shown in table 1.

The degree of final purification (C) was examined on disc electrophoresis. Fig. 4 shows the result of the experiment. Suger content of lectin was found to be galactose in comparison to the standard. Amino acid analysis was also carried out. Table II indicated the amino acid content of our lectin hydrolysate and that in literature.

Inbar *et al* (9) reported that castor bean lectin inhibits growth of tumor cells *in vive*. Guertler and Emmerich (10) treated the ficoll purified peripheral blood lymphocytes with florescein conjugated lectin from castor bean for 15 min., and the percentage of the cap forming cells were examined. The values of leukemia lymphocytes were reduced compared to the values obtained with normal lymphyocytes. The reduction was more than half in patients with acute and chronic myelogenous leukemia and immunoblastoma, it was ¼ in patients with chronic lymphocytic leukemia. Lectin also is used as a suger containing agent for isolation of polysacharides and glycoporteins (11). Studies of cell membrane using this agent give much information concerning membrane structure and biological functions (12). Therefore, Iranian castor bean can be used for preparation of lectin which may have some commercial and medical use.

Table II The amino acid content of purified lectin (A) in comparison to that in literature (B) (13).

Amino acid	A*	B*
Lysine	3.2	4.6
Histidine	2.0	1.7
Arginine	6.6	7.1
Aspartic acid	8.3	11.1
Threonine	6.0	7.3
Serine	6.6	7.2
Glutamic acid	12.6	8.4
Proline	7.1	5.4
Glysine	5.4	6.7
Alanine	5.4	5.5
Valine	2.9	5.8
Cystine	2.9	1.8
Methionine	12.2	1.23
Leucine	7.1	8.1
Isoleucine	1.7	7.2
Tyrosine	2.3	4.4
Phenylalanine	4.6	4.6

* Mol% = Amino acid molecules per 100 molecules of lectin.

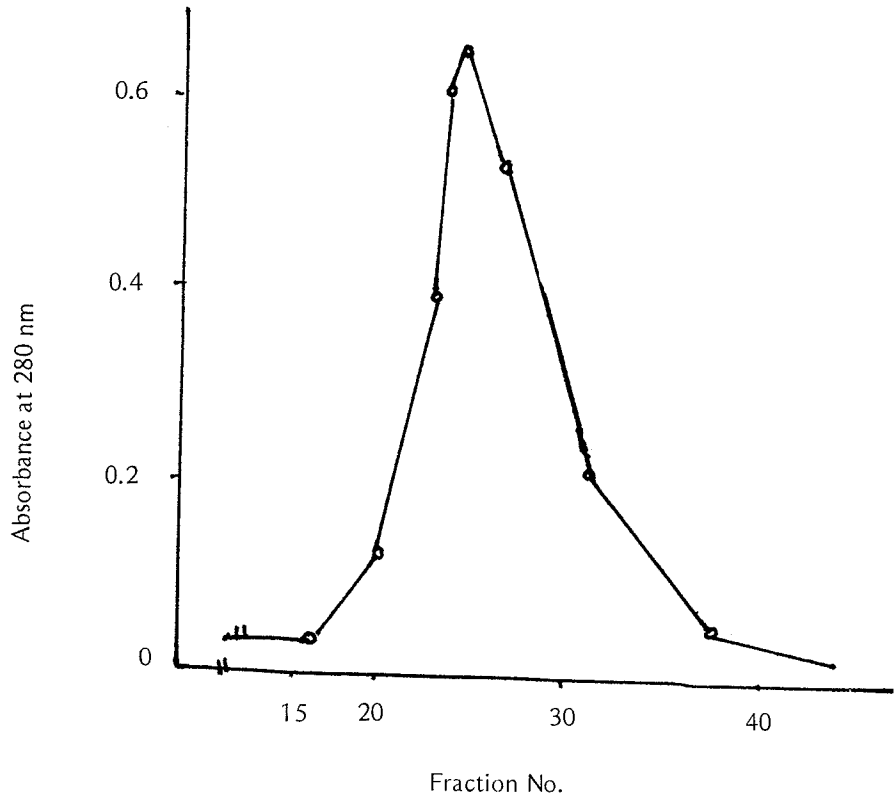

Fig. 1, Elution patern of lectin from agarose gel column.

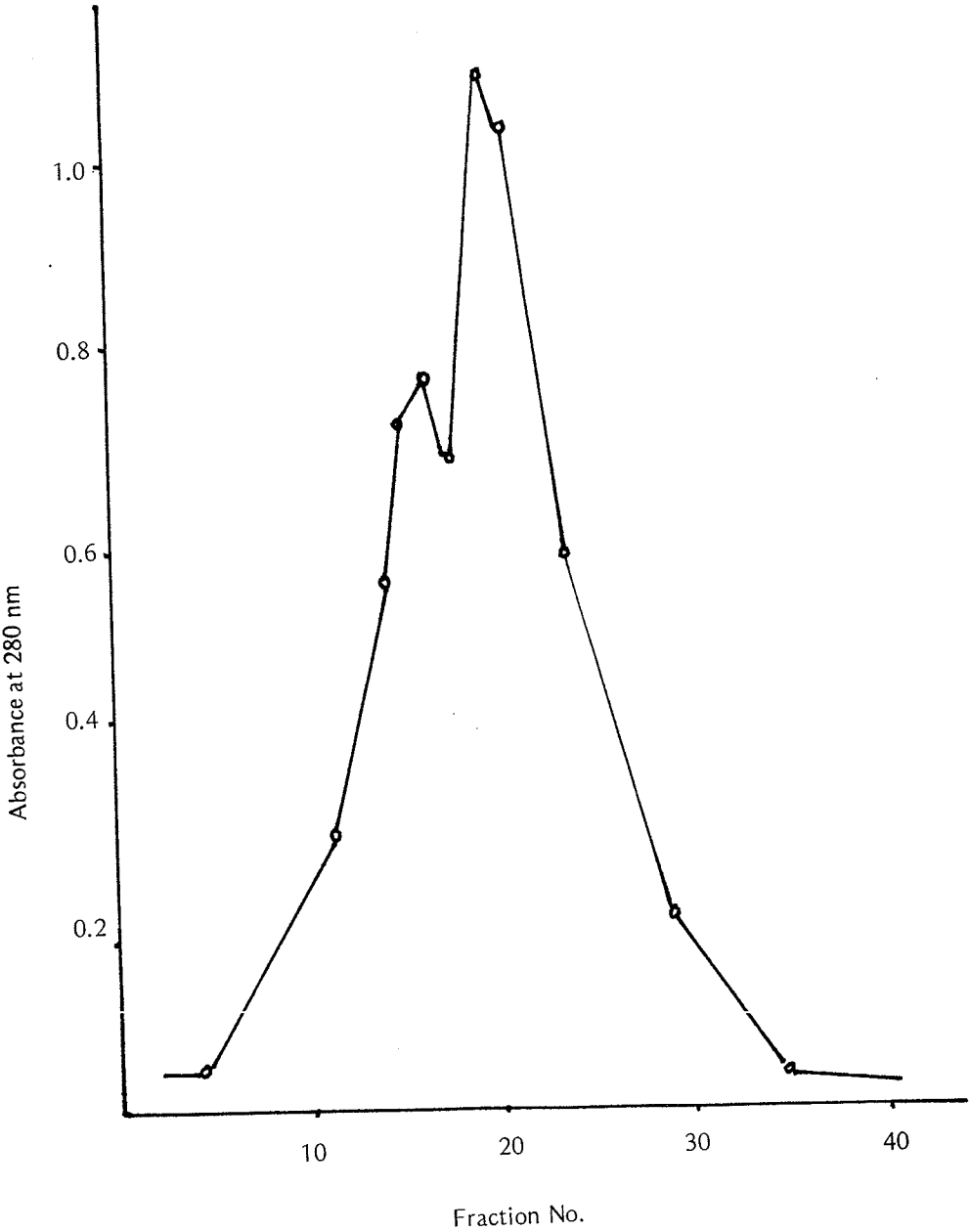

Fig. 2, Elution patern of lectin from Sephadex G-100

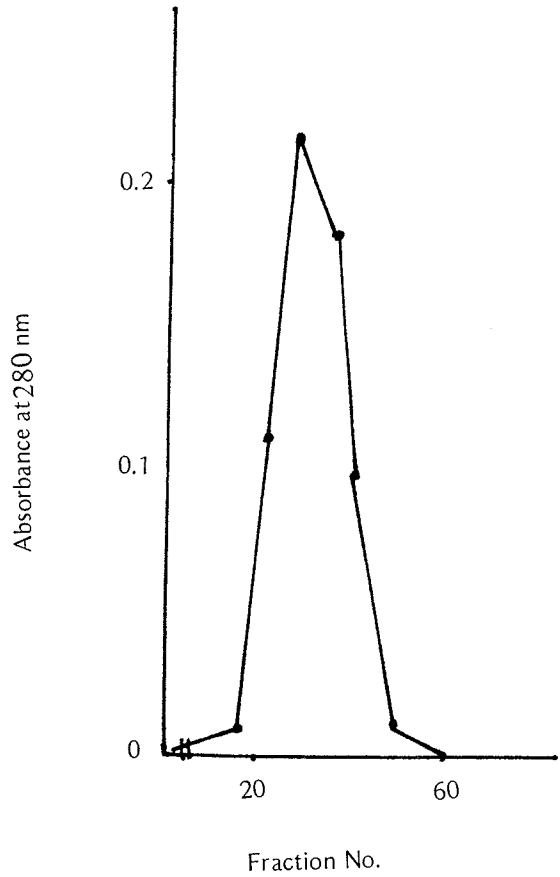

Fig. 3, Elution patern of lectin from Sephadex G-150

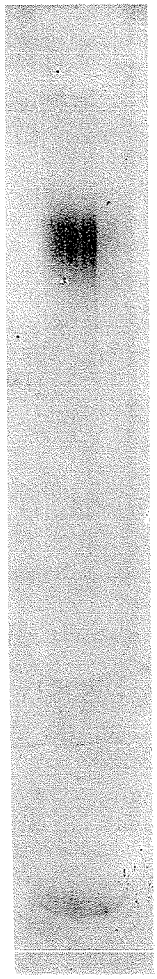

Fig. 4. Disc electrophoretic pattern of fractions from Sephadex G-100 column.

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