ISOLATION OF TWO FORMS OF A LECTIN FROM THE SEEDS OF *Datura innoxia*

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Two forms of a lectin, I_1 and I_2, with molecular weights of 150,000 and 300,000, respectively, have been isolated from the seeds of *Datura innoxia* by fractionation with ammonium sulfate, gel chromatography, and ion-exchange chromatography.

Lectins — carbohydrate-binding proteins — have been detected in many species of plants, in animal tissues, and in microorganisms. Lectins are revealed with the aid of a cell agglutination reaction, usually that of erythrocytes.

Agglutinating activity with respect to human erythrocytes of group A, B, and O was found by Boyd and Requera [1] in the seeds of *Datura innoxia*.

As we have reported previously, an extract of the seeds of this plant showed no specificity with respect to the mono- and disaccharides traditionally used, even at a concentration of the latter of 0.6 M [2]. Because of this, the use of affinity chromatography for the isolation of the lectin is difficult. We have performed the isolation and purification of the lectin by the methods generally adopted.

The lectin was extracted from a flour of the seeds with 15 mM phosphate buffer containing 0.9% of NaCl, pH 7.4 (PBS). Extraction with more concentrated salt solutions did not lead to an increase in hemagglutinating activity. The preliminary defatting of the flour had no influence on the yield of activity. The results of the isolation of the two forms of lectin from 80 g of seeds are given below:

	Total protein, mg	Yield %	Hemagglutinating activity		Degree of purification
			total, titer	specific, titer/mg	
Crude extract					
Fractionation with $(NH_4)_2SO_4$ 45%	4000	100	256	0,064	1
1. Precipitate	1 57	30	888	0,768	12
2. Supernatant	2843		—	—	—
Gel chromatography					
1. I_1	267 2	6 7	480	1 8	28
2. I_2	115,2	2,9	240	2,08	32,5

The agglutinin was precipitated from the extract at 45% saturation with ammonium sulfate. This led to a 12-fold increase in specific activity. The precipitate was suspended in 5 mM Na phosphate buffer, pH 7.4, and was dialyzed against deionized H_2O. On dialysis part of the lectin coprecipitated with the globulin. Its amount varied and depended on the conditions of performance of the dialysis. The supernatant solution was separated from the centrifugation precipitate. The supernatant consisted of a simpler mixture of proteins as compared with the precipitate. Disk electrophoresis in 7% PAAG, pH 8.9, gave three bands (Fig. 1).

The supernatant solution (after dialysis and centrifugation) was separated by gel chromatography into three homogeneous fractions, two of which possessed hemagglutinating activity. Figure 2 shows the distribution of activity relative to the protein fractions.

The active fractions I_1 and I_2 were collected and rechromatographed on a column of Sephadex G-200.

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Fig. 1

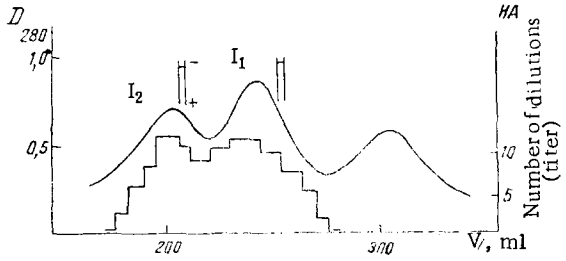

Fig. 2

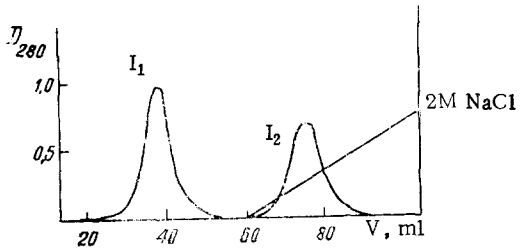

Fig. 3

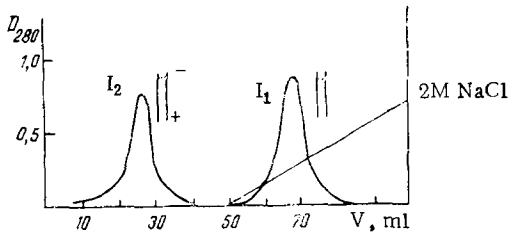

Fig. 4

On ion-exchange chromatography on CM-cellulose at pH 6.2, I_2 was eluted by the initial buffer (5 mM PBS) and I_1 when a linear gradient from 5 mM PB to 2 M NaCl in 5 mM PB, pH 2, was created (Fig. 3).

Fraction I_2 was deposited on DEAE-cellulose, pH 7.4, and was eluted as a symmetrical peak by a concentration gradient of NaCl. Fraction I_1 was eluted by the initial buffer (Fig. 4). The purified lectin fractions each gave a single band on disk electrophoresis in 7% PAAG, pH 8.9.

Thus, a simple three-stage method of isolation enabled us to obtain two forms of the *D. innoxia* lectin, I_1 and I_2, purified 28- and 32.5-fold, respectively, and amounting to ~7% (I_1) and ~3% (I_2) of the extractable protein. The total amount of lectin in the seeds was 0.5%.

The molecular weights of these substances were estimated with the aid of gel chromatography on Sephadex G-200 and were found to be 300,000 for I_2 and 150,000 for I_1. However, it was impossible to determine the molecular weights accurately, since gel chromatography could affect the dimensions of the molecule (the solutions of the lectins I_1 and I_2 possessed a high viscosity).

I_1 and I_2 agglutinate human erythrocytes of various blood groups with the same titer, and also those of the rabbit and the rat at a minimum concentration of 1 μg/ml. The lectin of *D. innoxia* agglutinates leukocytes at the same minimum concentration. It possesses no mitogenic activity. It is inhibited by chitin derivatives in a manner similar to the lectins of other species of the family *Solanaceae* [3, 4].

A comparative study of their structure and properties will permit an answer to the question of whether I_2 is a dimeric form of I_1 or if they are two independent lectins.

EXPERIMENTAL

The lectin was isolated by extraction from 80 g of ground seeds of *Datura innoxia* with 500 ml of PBS containing 0.9% of NaCl at 6°C with constant stirring for 20 h. The extract was separated from the cell residues by centrifugation at 3000 rpm for 15 min, and the supernatant was filtered through an absorbent cotton filter. The residue was reextracted with 300 ml of PBS. The protein was precipitated from the combined crude extract on 45% saturation

with ammonium sulfate at 6°C. The precipitate that had deposited after 18-20 h was collected by centrifugation at 12,000 rpm for 30 min. Then it was suspended in 100 ml of PBS and exhaustively dialyzed against deionized H_2O. The protein that precipitated on dialysis was separated off by centrifugation (12,000 rpm for 30 min). The precipitate and the supernatant solution were freeze-dried. The yields were 12 and 17% of the extracted protein, respectively.

Gel chromatography was performed on a column (3.0 × 50 cm) with Ultragel AcA34 in 5 mM Na phosphate buffer, pH 7.4, using 200-mg portions of protein in 1.5 ml of the initial buffer. Elution was performed at the rate of 22 ml/h, 5-ml fractions being collected. The titer of hemagglutinating activity was determined in each fraction.

Ion-exchange chromatography was performed on columns of CM-cellulose (2.0 × 12.0 cm) at pH 6.2 and of DEAE-cellulose (2.5 × 14 cm) at pH 7.45 with the switching in of a gradient from 5 mM PB to 2 mM NaCl in 5 mM PB. The amount of protein deposited on the CM-cellulose column was 80-100 mg, and the rate of elution was 36 ml/h. The amount of protein on the DEAE-cellulose column was 100 mg and the rate of elution here was 40 ml/h. In both cases, before being deposited on the column the protein was dialyzed against the corresponding buffer for 12 h.

Disk electrophoresis was performed in 7% polyacrylamide gel, pH 8.3, by a standard procedure [5]. Before being deposited on the gel, the samples of protein were incubated with 0.1% SDS for 30 min.

Protein concentrations were determined by the biuret method [5].

Hemagglutinating activity [6] was determined from the erythrocyte-agglutination reaction in 50-μl microtest-tubes. The maximum dilution of the lectin solution (a series of twofold dilutions was prepared) at which visual agglutination of the erythrocytes was just observed, was taken as the titer of, agglutinating activity.

Leukoagglutinating activity was determined in a similar manner to the hemagglutination reaction [7]. For the performance of the leukoagglutination reaction a solution of leukocytes containing 27 million cells in 1 ml of physiological solution was used. To micro test-tubes containing 50 μl of lectin solution were added 50-μl portions of the leukocyte suspension and they were placed in a shaking apparatus (100 vibrations per minute) for 30 min. The result of the reaction was observed under the microscope. The dilution at which 50% of the cells agglutinated was taken as the titer.

To determine mitogenic activity we used a solution of lectin with a concentration of 0.3 mg/ml. The lectin solution was first centrifuged at 18,000 rpm and was then sealed into a tube and autoclaved. To determine mitogenic activity, 0.05-0.1 ml portions of the solution were used. Mitogenic activity was determined by a known method [8].

SUMMARY

Two forms of a lectin I_1 and I_2 possessing multiple molecular weights have been isolated from the seeds of *Datura innoxia*. Lectins I_1 and I_2 are nonspecific to human erythrocytes on the A, B, O system and possess leukoagglutinating activity.

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