LECTIN FROM *Bryonia alba* ROOTS

L. M. Gogilashvili and E. P. Kemertelidze UDC 547.962.5

A method has been developed for isolating and purifying lectin from Bryonia alba *roots. Proteins with molecular weights from 45 to 67 kDa dominate in the partially purified lectin. The carbohydrate selectivity places the lectin in the galactose-specific group.*

Key words: lectin, *Bryonia alba* L. roots, hemagglutination, specific titer.

Lectins assumed a special role in biological research owing to their unique ability to bind selectively carbohydrates [1-3].

During a survey of lectins in the flora of Georgia, our attention was drawn to roots of *Bryonia alba* L. (Cucurbitaceae) [4]. The extract obtained from the roots agglutinized human group A erythrocytes with a titer of 1:256. The protein fraction containing the lectin was salted out with ammonium sulfate at 80% saturation after preliminary purification of the extract. The solid was dissolved in water, dialyzed, and further purified using ion-exchange chromatography on DEAE-cellulose with a stepwise gradient.

Protein fractions (Fig. 1) were obtained, of which only the fraction eluted by buffer III exhibited hemagglutination activity with native human erythrocytes. The fractions were combined according to the protein peaks and analyzed.

Table 1 presents the results. The specific titer of lectin after precipitation of the protein components from the initial extract with ammonium sulfate increases by 2.35 times; after chromatography on DEAE-cellulose, by 10 times. Therefore, the initial extract should be purified of a significant amount of accompanying ballast proteins. This increases the specific titer of the lectin.

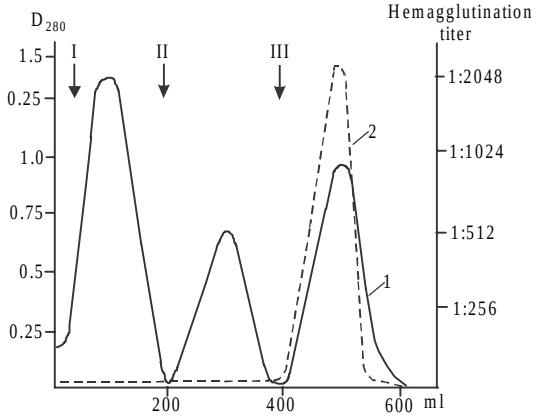

Fig. 1. Ion-exchange chromatography of *Bryonia alba* lectin on DEAE cellulose column (2.5 × 20 cm) equilibrated with buffer I. Elution: buffer I, 10 mM K_2HPO_4, pH 8.0 (I); buffer II, 100 mM K_2HPO_4, pH 5.3 (II), buffer III, 0.5 mM NaCl/200 mM K_2HPO_4, pH 5.0 (III). Protein content in samples (1) and hemagglutination activity (2).

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TABLE 1. Properties of *Bryonia alba* Lectin Fractions

Sample	Volume, ml	Conc., mg/ml	Total protein, mg	Agglutination of human group A erythrocytes	
				titer	specific titer*
Initial extract obtained from 100 g of raw material	1000	0.720	720	1:256	355.5
Fraction precipitated by ammonium sulfate	300	1.226	368	1:1024	835.24
Fractions obtained after ion-exchange chromatography with buffer					
I	200	1.0	200	Not active	-
II	200	0.3	60	Not active	-
III	200	0.54	108	1:2048	3792.6

*Specific titer = titer/protein concentration.

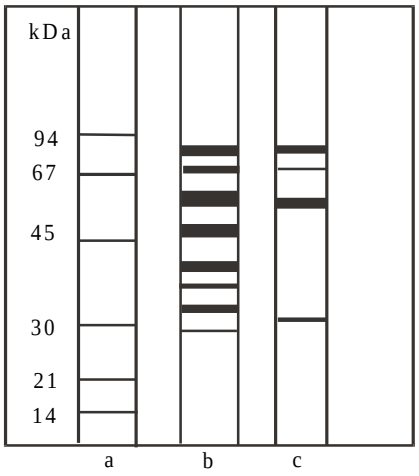

Fig. 2. Electrophoregrams of *Bryonia alba* lectin fractions and marker proteins in 15% PAAG, pH 8.9: phosphorylase B (94 kDa), bovine albumin (67 kDa), ovalbumin (45 kDa), carbohydrase (30 kDa), trypsin inhibitor (21 kDa), α-lactalbumin (14 kDa) (a), initial extract (b), lectin fraction after ion-exchange chromatography (c).

The composition of the proteins in the initial extract and fractions obtained after purification was studied using gel-electrophoresis in polyacrylamide gel (PAAG) and marker proteins. The initial extract of *Bryonia alba* roots consists of 10 protein components with molecular weights from 90 to 21 kDa (Fig. 2). After purification, the lectin fraction contains four bands, among which proteins with molecular weights from 45 to 67 kDa dominate.

Thus, the initial extract is significantly purified. However, the resulting lectin fraction is not homogeneous on electrophoresis.

Then the carbohydrate-binding specificity of partially purified lectin was studied using inhibition of hemagglutination and the following sugars: L-fucose, D-glucose, D-galactose, saccharose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-mannose, L-rhamnose, and D-fructose. Inhibition was observed for D-galactose, saccharose, and N-acetyl-D-galactosamine. The minimal concentrations necessary for inhibition were measured for these carbohydrates (mM): D-galactose (100),

saccharose (50), and N-acetyl-D-galactosamine (25). It can be seen that this lectin has 2-4 times greater affinity for N-acetyl-D-galactosamine than for saccharose and D-galactose.

Thus, partially purified lectin belonging to the galactose-specific group is obtained from *Bryonia alba* roots.

EXPERIMENTAL

Air-dried and ground roots of *Bryonia alba* that were collected in September-October near Tbilisi were extracted three times at room temperature with NaCl (0.9%, 1:10) with continuous stirring for 2 h. The extracts were combined and filtered. The resulting solution was acidified with 2 N HCl until the pH was 4.2. The precipitate was separated by centrifugation at 3000 rpm. The supernatant was neutralized with 5 N NaOH until the pH was 6.8. The protein fraction containing the lectin was salted out with ammonium sulfate at 80% saturation. The salting out time was 12 h. The mixture was centrifuged for 20 min at 3000 rpm. The precipitate was collected, pressed on a vacuum filter, dissolved in a five-fold excess of water, and dialyzed against water at 6°C for 24 h.

Erythrocyte preparation, hemagglutination in microtubes, and determination of carbohydrate specificity were performed according to the literature methods [5].

The protein content in the samples was determined spectrophotometrically from the UV absorption at 280 nm and by the Lowry method [6].

In chromatographic and electrophoretic studies we used DEAE-cellulose and a reagent set for disk-electrophoresis (Reanal, Hungary). Chromatography was performed according to recommendations of the sorbent manufacturer. Gel electrophoresis was carried out according to the literature method [7]

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